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SPECIFICATION FOR NONPROVISIONAL APPLICATION

BE IT KNOWN, that we, Wentao Zhang, a resident of Foster City, California, Sebastian Johannes Reinhard Liehr, a resident of East Palo Alto, California, Mark Douglas Velligan, a resident of Montara, California, Natalia B. Dyatkina, a resident of Mountain View, California, Janos Botyanszki, a resident of Cupertino, California, Dong-Fang Shi, a resident of San Mateo, California, Christopher Don Roberts, a resident of Belmont, California, Alexander Khorlin, a resident of Mountain View, California, Peter Harold Nelson, a resident of Los Altos, California, and Joseph Martin Muchowski, a resident of Sunnyvale, California, have invented new and useful improvements in:

NOVEL COMPOUNDS POSSESSING ANTIBACTERIAL, ANTIFUNGAL OR ANTITUMOR ACTIVITY

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NOVEL COMPOUNDS POSSESSING ANTIBACTERIAL, ANTIFUNGAL OR ANTITUMOR ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119(e) to U.S. Provisional Application Serial No. 60/214,478, which was filed on June 27, 2000, the disclosure of which is incorporated herein in its entirety.

BACKGROUND OF THE INVENTION

Field of Invention

The present invention provides novel compounds possessing one or more of the following activities: antibacterial, antifungal and antitumor activity. Pharmaceutical compositions containing these compounds, methods of making and methods for using these compounds are also provided.

State of the art

The binding of the antibacterial netropsin and distamycin to AT-rich sequences in the minor groove of double stranded DNA is a well studied phenomenon. Because such binding can be used to regulate DNA expression, e.g., by blocking and/or displacement of regulatory proteins, or by inhibiting the activity of enzymes acting on DNA, such as reverse transcriptase or topoisomerase, optimization of this binding has been the subject of numerous recent studies.

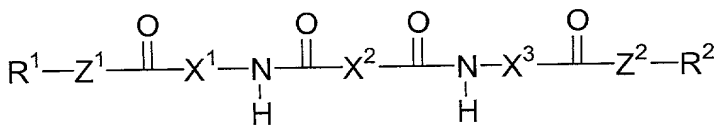
As described in a recent review by Bailly and Chaires (*Bioconj. Chem.* **9**(5):513-38, 1998), the pyrrolecarboxamide unit in netropsin and distamycin is actually about 20% longer than required to perfectly match the corresponding base pair sequence in the minor groove. Accordingly, in oligomeric analogs having multiple binding moieties, successive binding moieties can become out of phase with the base pairs of the minor groove. Several studies have therefore been directed to dimers of netropsin or distamycin containing different linkers,

in order to improve binding to longer target sequences. In these reports, effectiveness of various netropsin or distamycin dimers was determined, for example, in the inhibition of transcription by HIV-1 reverse transcriptase (M. Filipowsky *et al., Biochemistry* **35**:15397-410, 1996), inhibition of mammalian DNA topoisomerase I (Z. Wang *et al., Biochem. Pharmacol.* **53**:309-16, 1997), or inhibition of HIV 1 integrase (N. Neamati *et al., Mol. Pharmacol.* **54**:280-90, 1998).

Preferred linkers in these studies included p-phenylene, *trans*-vinyl, cyclopropyl, 3,5-pyridyl, and six- and eight-carbon aliphatic chains. Several of these linkers restrict rotation around the linking group, thus reducing the extent of purely monodentate binding (e.g. by only one netropsin moiety; see Bailly) which can occur with flexible linkers. However, Kissinger *et al.* (*Chem. Res. Toxicol.* **3**(2):162-8, 1990) reported that aryl-linked groups had reduced DNA binding affinity compared to alkyl and alkylene linkers, and Neamati *et al.* (cited above) reported that the *trans*-vinyl linked compound was many times more potent (in inhibiting HIV-1 integrase) than the "more rigid" cyclobutanyl and norbornyl linkers. It was suggested in Wang and in Bailly that, for certain applications, the more rigid linkers (cyclopropyl and p-phenylene) may not allow for optimal simultaneous (bidentate) binding of the two netropsin moieties flanking the linker. Therefore, it would be desirable to provide linkers which reduce monodentate binding but which provide suitable geometries for bidentate binding. In light of the increase of antibiotic /antifungal resistant organisms, there is a need to develop new compounds to treat diseases caused by these antibiotic /antifungal resistant organisms. The compounds of the present invention fulfill this need.

SUMMARY OF THE INVENTION

The present invention provides novel compounds which possess one or more of the following activities: antibacterial, antifungal and antitumor activity. Specifically, the compounds of this invention are represented in Formula (I) below:



(I)

wherein:

Z^1 and Z^2 are independently $-NR^3-$ (wherein R^3 is hydrogen or alkyl) or -O-;

R^1 and R^2 are independently substituted alkyl, substituted aryl, heteroaryl, or substituted heteroaryl provided that at least one of R^1 and R^2 is a group that can form a pharmaceutically acceptable acid addition salt;

R^3 is hydrogen, alkyl or R^3 and R^1 or R^2 together with the atoms to which they are attached form a heterocyclic ring;

X^2 is aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkenyl, alkynyl, cycloalkyl or heterocyclic;

X^1 and X^3 are independently aryl, substituted aryl, heteroaryl or substituted heteroaryl;

or a pharmaceutically acceptable acid addition salt thereof.

In a second aspect, this invention is directed to a method of treating bacterial and/or fungal infection(s), which method comprises administration of a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable an acid addition salt thereof.

In a third aspect, this invention is directed to a method of treating cancer through the inhibition of topoisomerase, which method comprises administration of a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable an acid addition salt thereof.

In a fourth aspect, this invention is directed to pharmaceutical compositions containing a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable acid addition salt thereof and a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates some representative compounds of this invention.

FIG. 2 illustrates further representative compounds of this invention.

FIGS. 3-4 illustrate even further representative compounds of this invention.

FIG. 5 illustrates examples of compounds possessing antibacterial activity.

FIG. 6 illustrates examples of compounds possessing antifungal activity.

Schemes 1-5 illustrate specific synthetic routes to compounds 7, 11-15, 20, 22 and 25, which are compounds of Formula (I).

Schemes 6-13 illustrate synthetic routes to various compounds of Formula (I).

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to novel compounds possessing one or more of the following activities: antibacterial, antifungal and antitumor activity. However, prior to describing this invention in further detail, the following terms will first be defined:

Unless otherwise stated, the following terms used in the specification and claims have the meanings given below:

"Alkyl" means a linear or branched saturated monovalent hydrocarbon radical of one to ten carbon atoms, preferably one to six carbon atoms, *e.g.*, methyl, ethyl, propyl, 2-propyl, *n*-butyl, *iso*-butyl, *tert*-butyl, pentyl, and the like.

"Substituted alkyl" means a linear or branched saturated monovalent hydrocarbon radical of one to ten carbon atoms, preferably one to six carbon atoms, which is substituted with 1 to 5 group(s), preferably 1 or 2 group(s), selected from the group consisting of hydroxy, alkoxy, acyl, acylamino, halo, thio, thioalkyoxy, amido, amino, mono or disubstituted amino, carboxy, amidino, guanidino, amidoxime, sulfonylamino, cycloalkyl, heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl and -$NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl). Representative examples include, but are not limited to, 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxy-1-hydroxymethylethyl, 2-hydroxy-2-hydroxymethylethyl, 1-hydroxymethylethyl, 3-hydroxybutyl, 2,3-dihydroxypropyl, 2,3-dihydroxypropyl, 2-hydroxy-1-methylpropyl, 2-methoxyethyl, 3-methoxypropyl, 2-acetylethyl, 3-acetylpropyl, 2-acetylaminoethyl, 3-acetylaminopropyl, 2-aminoethyl, 3-aminopropyl, dimethylaminoethyl, dimethylaminopropyl, 2-piperidin-1-ylethyl, 2-piperazin-1-ylethyl, 3-piperazin-1-ylpropyl, 3-piperazin-1-ylpropyl, 3-amidinopropyl, 3-guaindinopropyl, 2-imidazol-2-ylethyl, 3-imidazol-2-ylpropyl, and the like.

"Alkylene" means a linear or branched saturated divalent hydrocarbon radical of one to six carbon atoms, e.g., methylene, ethylene, 2,2-dimethylethylene, propylene, 2-methylpropylene, butylene, pentylene, and the like.

"Alkenyl" means a linear monovalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms, containing at least one double bond, e.g., ethenyl, propenyl, and the like.

"Substituted alkenyl" means an alkenyl radical, as defined herein, that is substituted with 1 to 3 group(s), preferably 1 or 2 group(s) selected from the group consisting of hydroxy,

alkoxy, acyl, acylamino, halo, amino, mono or disubstituted amino, carboxy, amidino, guanidino, sulfonylamino, heterocyclic, aryl, substituted aryl, heteroaryl, substituted heteroaryl and -$NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl).

"Alkynyl" means a linear monovalent hydrocarbon radical of two to six carbon atoms or a branched monovalent hydrocarbon radical of three to six carbon atoms, containing at least one triple bond, e.g., ethynyl, propynyl, and the like.

"Cycloalkyl" means a saturated monovalent cyclic hydrocarbon radical of three to six ring carbons, e.g., cyclopropyl, cyclopentyl, cyclohexyl, and the like.

"Substituted cycloalkyl" means a cycloalkyl radical as defined herein that is substituted independently with one, two or three substituents, preferably one or two substituents, selected from alkyl, alkoxy, substituted alkyl, acyl, acylamino, sulfonylamino, halo, nitro, cyano, amino, monosubstituted or disubstituted amino and -$NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl).

"Sulfonylamino" means a radical -$NRSO_2R'$ where R is hydrogen or alkyl and R' is alkyl, substituted alkyl, amino, monosubstituted amino, disubstituted amino, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroaralkyl, and substituted heteroaralkyl, e.g., methylsulfonylamino, benzylsulfonylamino, N-methylaminosulfonylamino, and the like.

"Alkoxy " means a radical -OR where R is an alkyl as defined above e.g., methoxy, ethoxy, propoxy, butoxy and the like.

"Acyl" means a radical –C(O)R, where R is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroaralkyl, substituted heteroaralkyl, heterocyclic, and heterocyclicalkyl group as defined herein. Representative examples include, but are not limited to formyl, acetyl, benzoyl, benzylcarbonyl, glycyl and the like.

"Acylamino" means a radical –NR'C(O)R, where R' is hydrogen or alkyl, and R is hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroaralkyl, substituted heteroaralkyl, heterocyclic, and heterocyclicalkyl group as defined herein. Representative examples include, but are not limited to formylamino, acetylamino, benzoylamino, benzylcarbonylamino, and the like. Preferred acylamino groups include the following: -$NHC(O)CH(NH_2)CH_3$; -

$NHC(O)CH(NH_2)-(CH_2)_3-NH-C(NH)NH_2$; $-NHC(O)CH(NH_2)-CH_2-C(O)NH_2$; $-NHC(O)CH(NH_2)-CH_2-CO_2H$; $-NHC(O)CH(NH_2)-CH_2-SH$; $-NHC(O)CH(NH_2)-(CH_2)_2-C(O)NH_2$; $-NHC(O)CH(NH_2)-(CH_2)_2-CO_2H$; $-NHC(O)CH_2-NH_2$; $-NHC(O)CH(NH_2)-CH_2-(C_3H_2N_2)$; $-NHC(O)CH(NH_2)-CH(CH_3)CH_2CH_3$; $-NHC(O)CH(NH_2)-CH_2CH(CH_3)_2$; $-NHC(O)CH(NH_2)-(CH_2)_4-NH_2$; $-NHC(O)CH(NH_2)-(CH_2)_2-SCH_3$; $-NHC(O)CH(NH_2)-CH_2Ph$; $-NHC(O)CH(NH_2)-(C_4H_8N)$; $-NHC(O)CH(NH_2)-CH_2OH$; $-NHC(O)CH(NH_2)-CH(OH)CH_3$; $-NHC(O)CH(NH_2)-CH_2-(C_8H_6N)$; $-NHC(O)CH(NH_2)-CH_2$-Ph-*p*-OH; and, $-NHC(O)CH(NH_2)-CH(CH_3)_2$.

"Monosubstituted amino" means a radical -NHR where R represents an alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroaralkyl, substituted heteroaralkyl, heterocyclic, and heterocyclicalkyl group as defined herein. Representative examples include, but are not limited to methylamino, ethylamino, phenylamino, benzylamino, and the like.

"Disubstituted amino" means a radical –NRR' where R and R' are independently selected from the group consisting of alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroaralkyl, substituted heteroaralkyl, heterocyclic, and heterocyclicalkyl group as defined herein. Representative examples include, but are not limited to dimethylamino, diethylamino, ethylmethylamino, diphenylamino, dibenzylamino, and the like.

"Halo" means fluoro, chloro, bromo, or iodo, preferably fluoro and chloro.

"Haloalkyl" means alkyl substituted with one or more same or different halo atoms, e.g., $-CH_2Cl$, $-CF_3$, $-CH_2CF_3$, $-CH_2CCl_3$, and the like.

"Aryl" means a monovalent monocyclic, bicyclic or tricyclic aromatic hydrocarbon radical of 6 to 14 ring atoms e.g., phenyl, naphthyl, or anthryl.

"Substituted aryl" means an aryl ring as defined above which is substituted independently with one, two or three substituents, preferably one or two substituents, selected from alkyl, alkoxy, aryloxy, substituted alkyl, acyl, acylamino, sulfonylamino, halo, nitro, cyano, amino, monosubstituted or disubstituted amino and $-NRSO_2NR'R"$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl).

"Heteroaryl" means a monovalent monocyclic, bicyclic or tricyclic radical of 5 to 12 ring atoms having at least one aromatic ring containing one, two, three or four ring heteroatoms selected from N, O, or S, the remaining ring atoms being C, with the

understanding that the attachment point of the heteroaryl radical will be on an aromatic ring. More specifically the term heteroaryl includes, but is not limited to, pyridyl, furanyl, thienyl, thiazolyl, tetrazolyl, isothiazolyl, triazolyl, imidazolyl, isoxazolyl, pyrrolyl, pyrazolyl, pyrimidinyl, benzofuranyl, isobenzofuranyl, benzothiazolyl, benzoisothiazolyl, benzotriazolyl, indolyl, isoindolyl, benzoxazolyl, quinolyl, tetrahydroquinolinyl, isoquinolyl, benzimidazolyl, benzisoxazolyl or benzothienyl.

"Substituted heteroaryl" means a heteroaryl ring as defined above which is substituted independently with one, two or three substituents, preferably one or two substituents, selected from alkyl, alkoxy, aryloxy, substituted alkyl, acyl, acylamino, sulfonylamino, halo, nitro, cyano, amino, monosubstituted or disubstituted amino and $-NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl).

"Aralkyl", "heteroaralkyl", "substituted aralkyl", "substituted heteroaralkyl", means a radical $-R^aR^b$ where R^a is an alkylene group and R^b is a aryl or substituted aryl, heteroaryl or substituted heteroaryl group as defined herein, *e.g.*, benzyl, pyridin-3-ylmethyl, imidazolylethyl, pyridinylethyl, 3-(benzofuran-2-yl)propyl, and the like.

"Heterocyclic" means a saturated non-aromatic cyclic radical of 5 to 8 ring atoms in which one or two ring atoms are heteroatoms selected from NR (where R is independently hydrogen, alkyl, or heteroalkyl), O, or $S(O)_n$ (where n is an integer from 0 to 2), the remaining ring atoms being C, where one or two C atoms may optionally be replaced by a carbonyl group. The heterocyclic ring may be optionally substituted independently with one, two, or three substituents selected from alkyl, alkoxy, substituted alkyl, acyl, acylamino, sulfonylamino, halo, nitro, cyano, amino, monosubstituted or disubstituted amino and $-NRSO_2NR'R''$ (where R is hydrogen or alkyl and R' and R" are independently hydrogen, alkyl, haloalkyl, aryl, substituted aryl, heteroaryl, and substituted heteroaryl). More specifically the term heterocyclic includes, but is not limited to, tetrahydropyranyl, 2,2-dimethyl-1,3-dioxolane, piperidino, N-methylpiperidin-3-yl, piperazino, N-methylpyrrolidin-3-yl, 3-pyrrolidino, morpholino, thiomorpholino, thiomorpholino-1-oxide, thiomorpholino-1,1-dioxide, pyrrolinyl, imidazolinyl, and the derivatives thereof.

"Heterocyclicalkyl" means a radical $-R^aR^b$ where R^a is an alkylene group and R^b is a heterocyclic group as defined herein, *e.g.*, tetrahydropyran-2-ylmethyl, 4-methylpiperazin-1-ylethyl, 3-piperidinylmethyl, 2,2-dimethyl-1,3-dioxoxolan-4-ylmethyl, benzyl, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "heterocyclic group optionally mono- or di- substituted with an alkyl group" means that the alkyl may but need not be present, and the description includes situations where the heterocyclic group is mono- or disubstituted with an alkyl group and situations where the heterocyclic group is not substituted with the alkyl group.

"Hydroxy or amino protecting group" refers to those organic groups intended to protect oxygen and nitrogen atoms against undesirable reactions during synthetic procedures . Suitable oxygen and nitrogen protecting groups are well known in the art e.g., trimethylsilyl, dimethyl-*tert*-butylsilyl, benzyl, benzyloxy-carbonyl (CBZ), *tert*-butoxycarbonyl (Boc), trifluoroacetyl, 2-trimethylsilylethanesulfonyl (SES), and the like. Others can be found in the book by T. W. Greene and G. M. Wuts, *Protecting Groups in Organic Synthesis*, Third Edition, Wiley, New York, 1999, and references cited therein.

Amino acid refers to any of the naturally occurring amino acids, as well as synthetic analogs (*e.g.*, D-stereoisomers of the naturally occurring amino acids, such as D-threonine) and derivatives thereof. α-Amino acids comprise a carbon atom to which is bonded an amino group, a carboxyl group, a hydrogen atom, and a distinctive group referred to as a "side chain". The side chains of naturally occurring amino acids are well known in the art and include, for example, hydrogen (e.g., as in glycine), alkyl (e.g., as in alanine, valine, leucine, isoleucine, proline), substituted alkyl (e.g., as in threonine, serine, methionine, cysteine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, and lysine), arylalkyl (e.g., as in phenylalanine and tryptophan), substituted arylalkyl (e.g., as in tyrosine), and heteroarylalkyl (e.g., as in histidine). Unnatural amino acids are also known in the art, as set forth in, for example, Williams (ed.), Synthesis of Optically Active .alpha.-Amino Acids, Pergamon Press (1989); Evans et al., J. Amer. Chem. Soc., 112:4011-4030 (1990); Pu et al., J. Amer. Chem. Soc., 56:1280-1283 (1991); Williams et al., J. Amer. Chem. Soc., 113:9276-9286 (1991); and all references cited therein. The present invention includes the side chains of unnatural amino acids as well.

Compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed "isomers". Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers".

Stereoisomers that are not mirror images of one another are termed "diastereomers"

and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a "racemic mixture".

The compounds of this invention may possess one or more asymmetric centers; such compounds can therefore be produced as individual I- or (S)- stereoisomers or as mixtures thereof. For example, if the R^1 substituent in a compound of formula (I) is 2-hydroxyethyl, then the carbon to which the hydroxy group is attached is an asymmetric center and therefore the compound of Formula (I) can exist as an I- or (S)-stereoisomer. Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art (*see* discussion in Chapter 4 of "Advanced Organic Chemistry", 4th edition J. March, John Wiley and Sons, New York, 1992).

A "pharmaceutically acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes an excipient that is acceptable for veterinary use as well as human pharmaceutical use. A "pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

"Pharmaceutically acceptable acid addition salts" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

Groups which form pharmaceutically acceptable acid addition salts include amines, hydrazines, amidines, guanidines, substituted aryl/heteroaryl and substituted alkyl groups that carry at least a nitrogen bearing substitutent such as amino, uanidine, amidino, uanidine and the like.

Amine groups are represented by the formula –NR'R" where R' and R" are independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heterocyclic, heteroaryl, substituted heteroaryl, and where R' and R", together with the nitrogen to which they are attached, form a heterocyclic or heteroaryl group.

Hydrazines are represented by the formula –NHNR'R" where R' and R" are independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heterocyclic, heteroaryl, substituted heteroaryl, and where R' and R", together with the nitrogen to which they are attached, form a heterocyclic or heteroaryl group.

Amidino groups are represented by the formula –C(=NH) NR'R" where R' and R" are independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heterocyclic, heteroaryl, substituted heteroaryl, and where R' and R", together with the nitrogen to which they are attached, form a heterocyclic or heteroaryl group.

Guanidino groups is represented by the formula –NHC(=NH)NR'R" where R' and R" are independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, heterocyclic, heteroaryl, substituted heteroaryl, and where R' and R", together with the nitrogen to which they are attached, form a heterocyclic or heteroaryl group.

A compound of Formula (I) may act as a pro-drug. Prodrug means any compound which releases an active parent drug according to Formula (I) *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of a compound of Formula (I) are prepared by modifying functional groups present in the compound of Formula (I) in such a way that the modifications may be cleaved *in vivo* to release the parent compound. Prodrugs include compounds of Formula (I) wherein a hydroxy, amino, or sulfhydryl group in compound (I) is bonded to any group that may be cleaved *in vivo* to regenerate the free hydroxyl, amino, or sulfhydryl group, respectively. Examples of prodrugs include, but are not limited to esters

(e.g., acetate, formate, and benzoate derivatives), carbamates (e.g., N,N-dimethylamino-carbonyl) of hydroxy functional groups in compounds of Formula (I), and the like.

"Treating" or "treatment" of a disease includes:

(3) preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a mammal that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease,

(3) inhibiting the disease, i.e., arresting or reducing the development of the disease or its clinical symptoms, or

(3) relieving the disease, i.e., causing regression of the disease or its clinical symptoms.

A "therapeutically effective amount" means the amount of a compound that, when administered to a mammal for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

"Anti-fungal" or "anti-bacterial" means that growth of the fungus or bacterial is inhibited or stopped.

"Anti-tumor" means the compound has the property of inhibiting the growth of tumor cells. Preferably, when the compound is contacted with a tumor cell line at a concentration of 100 μm, growth of the tumor cells is 32 % or less as that of a no growth control.

"Bacteriostatic" means the compound has the property of inhibiting bacterial or fungal multiplication, wherein multiplication resumes upon removal of the active compound. For a bacteriostatic compound, its minimum bacteriocidal concentration (MBC) is greater than 4x its minimum inhibitory concentration (MIC).

"Bacteriocidal" or "fungicidal" means that the compound has the property of killing bacteria or fungi. Bacteriocidal/fungicidal action differs from bacteriostasis or fungistasis only in being irreversible. For example, the "killed" organism can no longer reproduce, even after being removed form contact with the active compound. In some cases, the active compound causes lysis of the bacterial or fungal cell; in other cases the bacterial or fungal cell remains intact and may continue to be metabolically active. A bacteriocidal compound exhibits a MBC that is less than 4x its MIC. Similarly, a fungicidal compound exhibits a minimum fungicidal concentration (MFC) that is less than 4x its MIC.

"Minimum inhibitory concentration" or "MIC" refers to the minimum concentration of a compound necessary to completely inhibit growth of the organism tested. Compounds of

this invention having an MIC of at least 1 mM are active in the assays described in the examples below. In a preferred compounds have an MIC of 500 μM, and even more preferably an MIC of 100μM.

"dsDNA" means double stranded DNA.

PREFERRED EMBODIMENTS

While the broadest definition of this invention is set forth in the Summary of the Invention, certain compounds of Formula (I) are preferred.

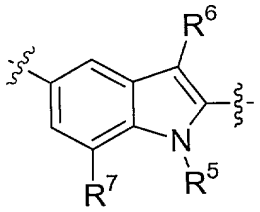
(A) A preferred group of compounds is that wherein Z^1 and Z^2 are –NH-.

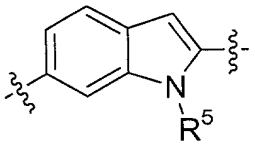
(B) Another preferred group of compounds is that wherein X^2 is aryl, substituted aryl, heteroaryl or substituted heteroaryl.

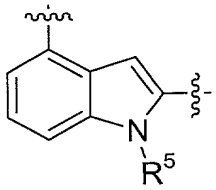
(C) Another preferred group of compounds is that wherein X^1 and X^3 are independently heteroaryl or substituted heteroaryl.

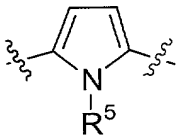
(D) Another preferred group of compounds is that wherein R^1 and R^2 are independently substituted alkyl groups.

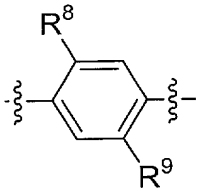
(E) Another preferred group of compounds is that wherein X^2 is an aryl, substituted aryl, heteroaryl or substituted heteroaryl moiety selected from a group consistingof the following moieties:

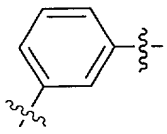


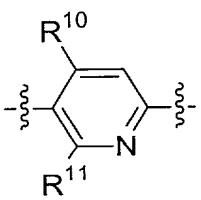


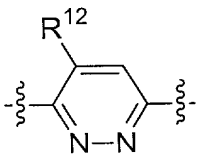


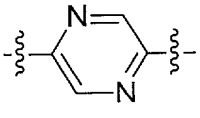


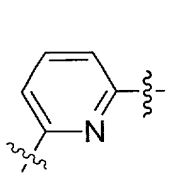
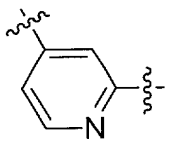
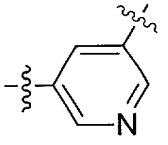
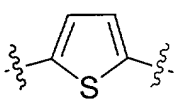
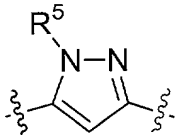
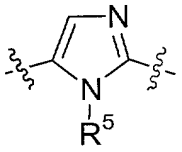
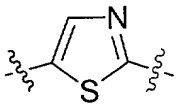
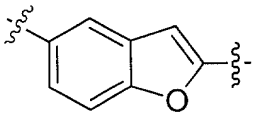
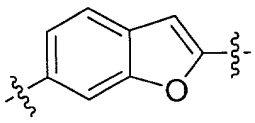
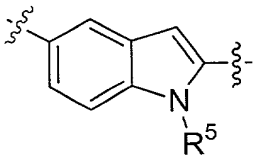
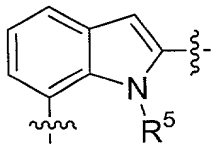
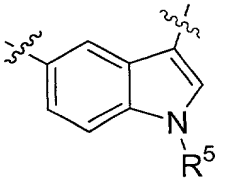
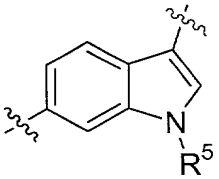
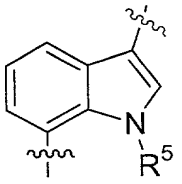
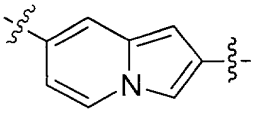


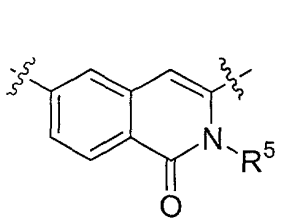
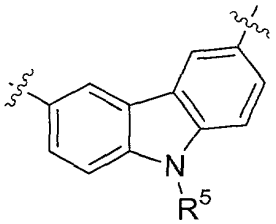
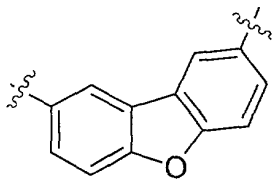
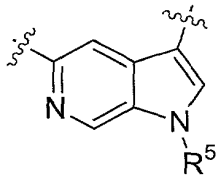
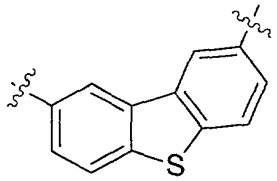
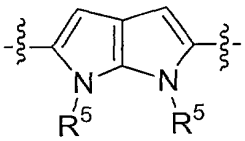








wherein,

R^5 is hydrogen, alkyl or substituted alkyl;

R^6 is hydrogen, alkyl, halo or alkoxy;

R^7 is hydrogen, alkyl or halo;

R^8 is hydrogen, alkyl, substituted alkyl, alkoxy or halo;

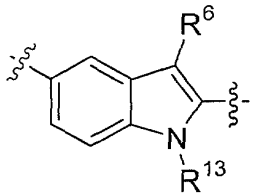
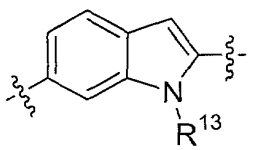
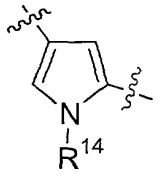
R^9 is hydrogen, alkyl, substituted alkyl, alkoxy, nitro or halo;

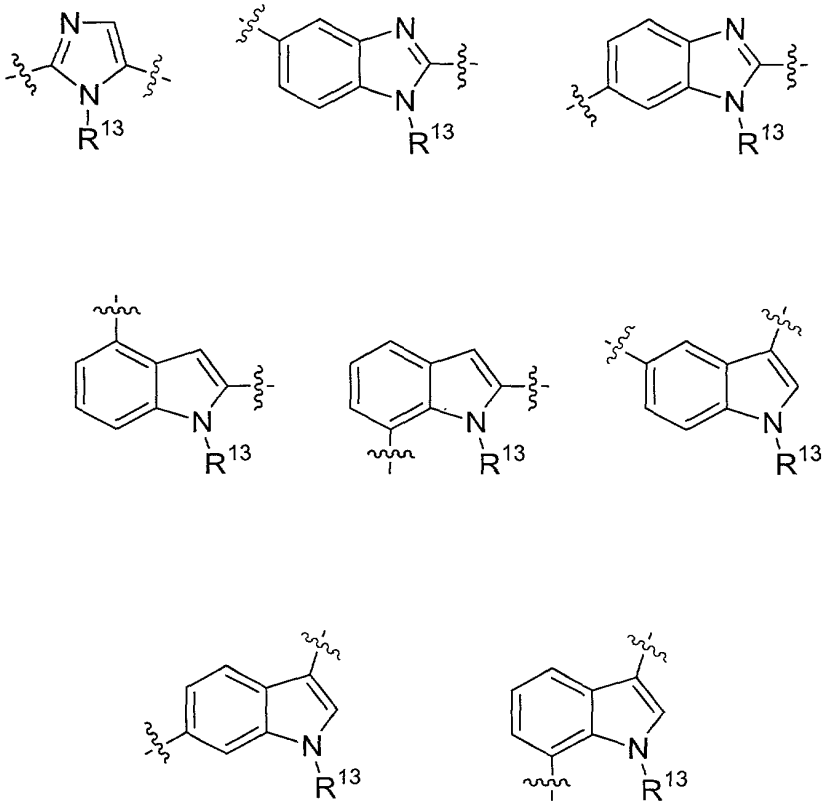
R^{10} is hydrogen or alkyl;

R^{11} is hydrogen or alkyl; and,

R^{12} is hydrogen or alkyl.

(F) Another preferred group of compounds is that wherein X^1 and X^3 are heteroaryl or substituted heteroaryl moieties independently selected from a group consisting of the following moieties:



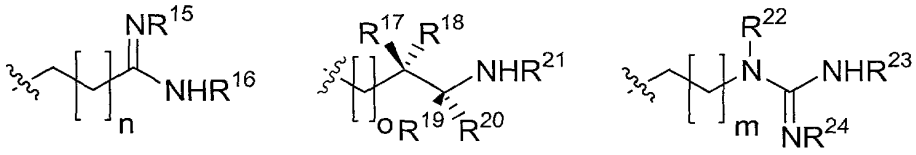
wherein

R^6 is hydrogen, alkyl, halo or alkoxy;

R^{13} is hydrogen or alkyl; and,

R^{14} is hydrogen, alkyl, substituted alkyl or aralkyl.

(G) Another preferred group of compounds is that wherein R^1 and R^2 are substituted alkyl moieties independently selected from the group consisting of the following moieties:



wherein

R^{15} is hydrogen, hydroxyl, alkoxyl, alkyl, cycloalkyl or R^{15} and R^{16} together with the atoms to which they are attached form a heterocyclic ring;

R^{16} is hydrogen, alkyl, hydroxyl or cycloalkyl;

R^{17}, R^{18}, R^{19} and R^{20} are independently hydrogen or alkyl;

R^{21} is hydrogen alkyl, substituted alkyl, cycloalkyl or acyl;

R^{22} is hydrogen or alkyl, or R^{22} and R^{23} together with the atoms to which they are attached form a heterocyclic ring, or R^{22} and R^{24} together with the atoms to which they are attached form a heterocyclic ring.

R^{23} is hydrogen, alkyl, hydroxyl, cycloalkyl or R^{23} and R^{24} together with the atoms to which they are attached form a heterocyclic ring;

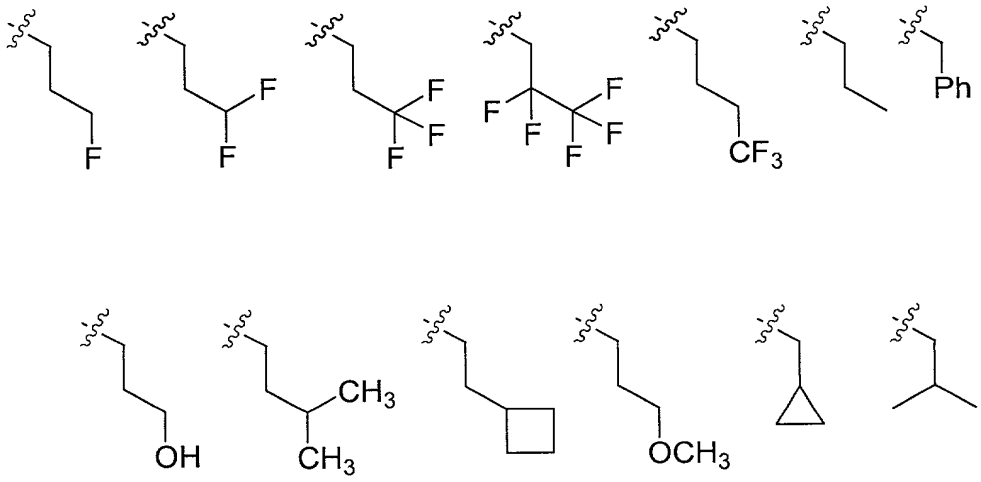
R^{24} is hydrogen, hydroxyl or alkyl;

m is 1, 2 or 3;

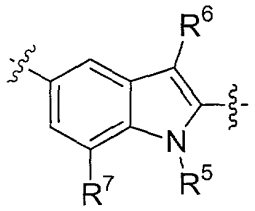
n is 1, 2 or 3; and,

o is 0, 1, 2 or 3.

(H) Another preferred group of compounds is that wherein R^{14} is an alkyl, substituted alkyl or aralkyl moiety, and wherein the moiety is selected from a group consisting of the following moieties:



(I) Another preferred group of compounds is that wherein X^2 is



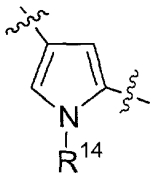
wherein,

R^5 is hydrogen, alkyl or substituted alkyl;

R^6 is hydrogen, alkyl, halo or alkoxy; and,

R^7 is hydrogen, alkyl or halo.

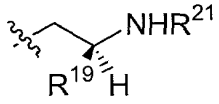
(J) Another preferred group of compounds is that wherein X^1 and X^3 are both

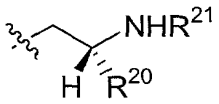


wherein

R^{14} is hydrogen, alkyl, substituted alkyl or aralkyl.

(K) Another preferred group of compounds is that wherein R^1 and R^2 are independently of one of the following structures:



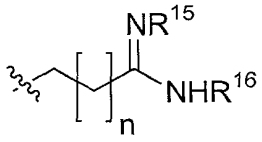


wherein

R^{19} and R^{20} are independently hydrogen or alkyl; and,

R^{21} is hydrogen, alkyl or acyl.

(L) Another preferred group of compounds is that wherein R^1 and R^2 are of the following structure:

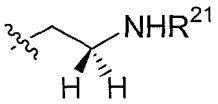


wherein

R^{15} and R^{16} are hydrogen, and,

n is 1 or 2.

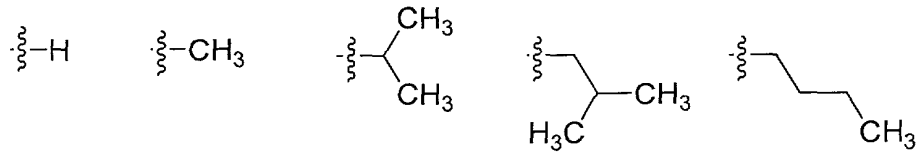
(M) Another preferred group of compounds is that wherein R^1 and R^2 are of the following structure:

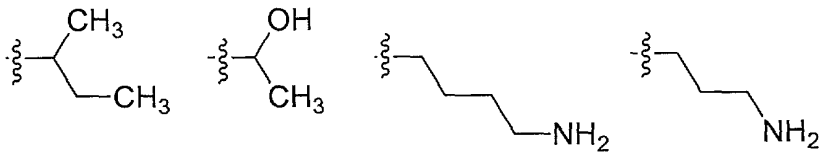


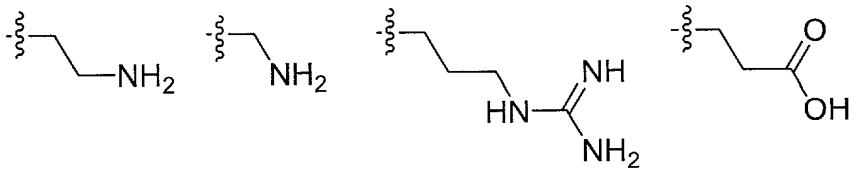
wherein

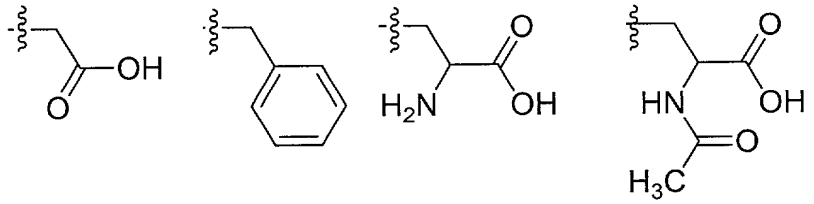
R^{21} is an alkyl group selected from a group consisting of methyl, ethyl, $-CH_2CH_2OH$, $-CH_2CH_2OAc$ and propyl, or an acyl moiety of the structure $-C(O)C(R^{25})(R^{26})H$;

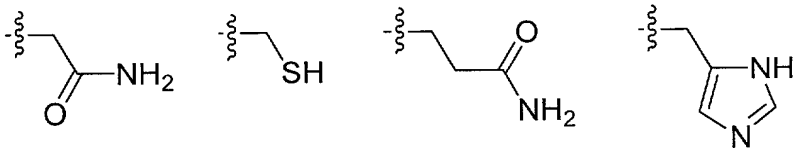
R^{25} is a substituent selected from a group consisting of the following substituents:

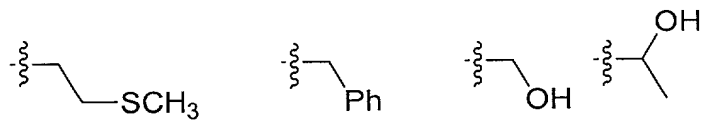


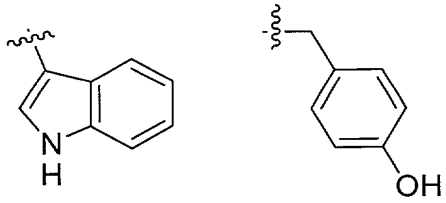




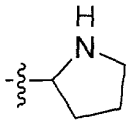








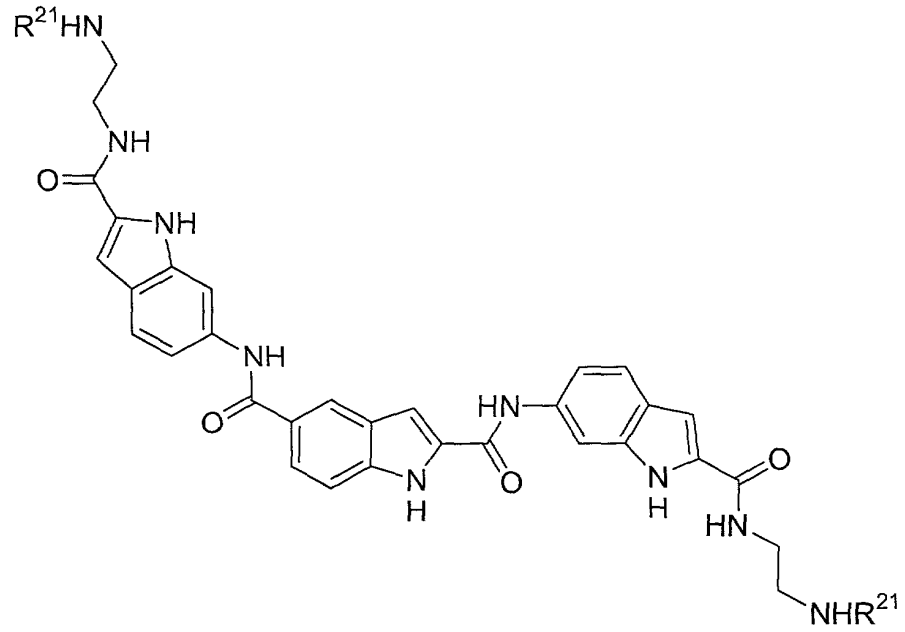
or R^{25} and R^{26} together with the atom to which they are attached form a heterocyclic ring of the following structure:



and,

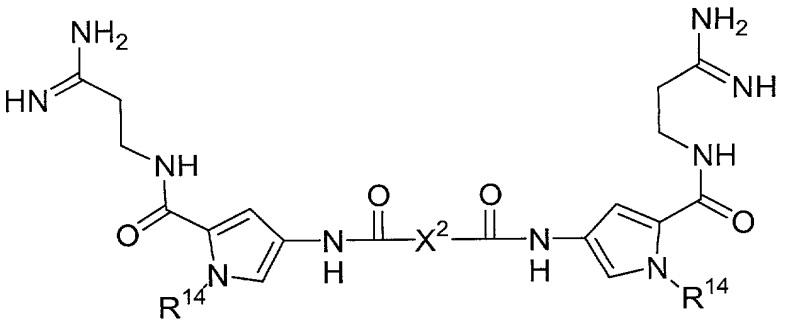
R^{26} is a substituent selected from a group consisting of the following substituents: -H, $-NH_2$ and $-NHCH_3$.

(N) Another preferred group of compounds is that wherein the compound of formula (I) is of the following structure:



wherein R^{21} is an alkyl group or an acyl group.

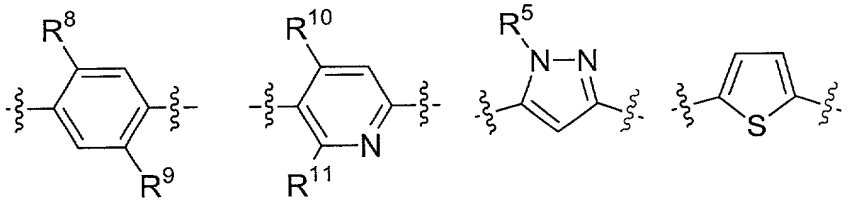
(O) Another preferred group of compounds is that wherein the compound of formula (I) is of the following structure:

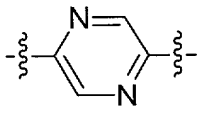


wherein

R^{14} is hydrogen, $-CH_2CH_2CH(CH_3)_2$ or $-CH_2(C_3H_5)$; and,

X^2 is a moiety selected from a group consisting of the following moieties:





GENERAL SYNTHETIC SCHEME

Compounds of this invention can be made by the methods depicted in the reaction schemes shown below.

The starting materials and reagents used in preparing these compounds are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemie, or Sigma (St. Louis, Missouri, USA) or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-15 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989), Organic Reactions, Volumes 1-40 (John

Wiley and Sons, 1991), March's Advanced Organic Chemistry, (John Wiley and Sons, 5th Edition), and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989). These schemes are merely illustrative of some methods by which the compounds of this invention can be synthesized, and various modifications to these schemes can be made and will be suggested to one skilled in the art having referred to this disclosure. (make sure lastest volumes included, any better book and suppliers of sm)

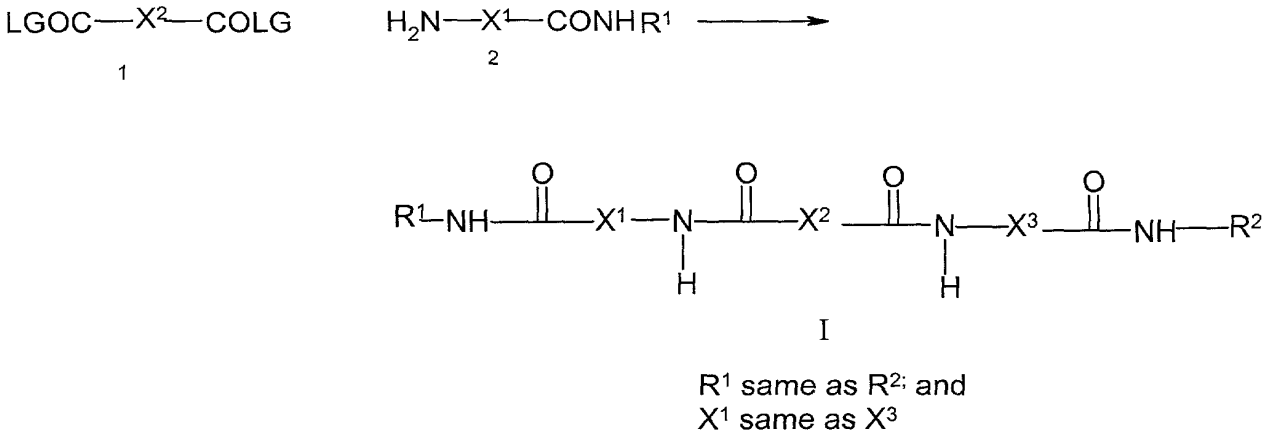
The starting materials and the intermediates of the reaction may be isolated and purified if desired using conventional techniques, including but not limited to filtration, distillation, crystallization, chromatography, and the like. Such materials may be characterized using conventional means, including physical constants and spectral data.

Preparation of compounds of Formula (I)

Schemes A and B describe alternative methods to prepare the compounds of Formula (I).

Compounds of Formula (I) where Z^1 and Z^2 are –NH-; R^1 and R^2 and X^1 and X^2 are as defined in the Summary of the Invention and are the same can be prepared as shown in Scheme A below.

Scheme A



A compound of Formula I wherein Z^1 and Z^2 are –NH-; R^1 and R^2 and X^1 and X^2 are as defined in the Summary of the Invention and are the same can be prepared in one step by

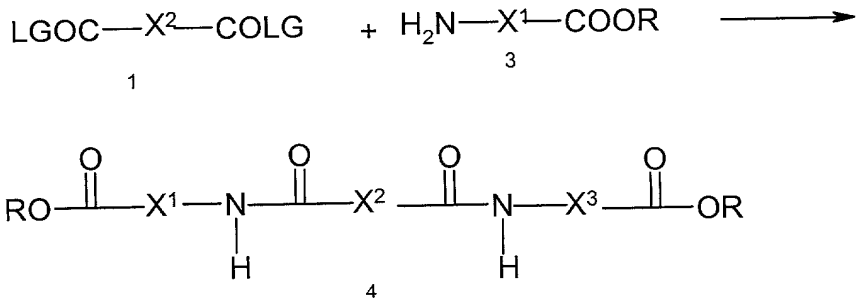
reacting a dicarboxylic acid derivative 1 (wherein LG is a suitable leaving group such as halo, pentafluorophenyloxy, and the like) with at least two equivalent of an amine of formula 2. The reaction is typically carried out in a polar organic solvent such as dimethylformamide, tetrahydrofuran, and the like and at an ambient temperature. It will be recognized by a person skilled in the art that if the leaving group is halo, then the reaction will be conducted in the presence of a non-nucleophilic base such as triethylamine and the like.

Compounds of formula 1 and 2 are commercially available from vendors such as Aldrich, Sigma, etc. Alternately these compounds can be prepared by methods well known in the art. For example, compounds of formulae 1 and 2 can be prepared by the procedure illustrated in Scheme 1 and described in detail in Example 1 below.

Additionally, it will be readily apparent to a person skilled in the art that a compound of Formula I where Z^1 and Z^2 are –O- can be prepared by following the above procedure but substituting the amino group in compound 2 with a hydroxy group.

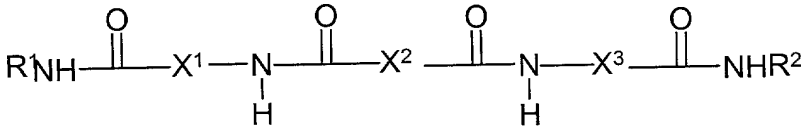
Alternatively, compounds of Formula (I) where Z^1 and Z^2 are –NH-; R^1 and R^2 and X^1 and X^2 are as defined in the Summary of the Invention and are the same can be prepared as shown in Scheme B below.

Scheme B



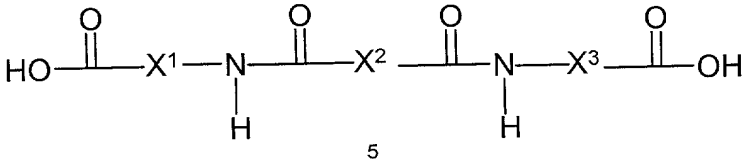
1 + 3 → 4

4 → (a) R^1NH_2 → I; (b) → 5

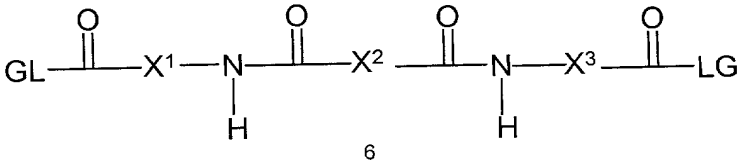


I

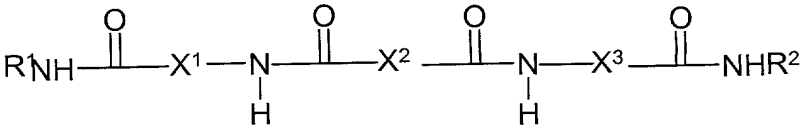
R^1 same as $R^{2;}$ and
X^1 same as X^3



5 → 6



6 → R^1NH_2 → I



I

R^1 same as $R^{2;}$ and
X^1 same as X^3

Reaction of a compound of formula 1 with an amino ester of formula 3 under conditions described in Scheme A above provides a diester compound of formula 4. Compound 4 is then converted to a compound of Formula I by following the procedures illustrated in method (a) or (b) above. In method (a), the diester 4 is treated with at least two equivalents of an amine of formula R^1NH_2 to provide a compound of Formula (I). The reaction is carried out between 40-60 °C and in a polar organic solvent such as dimethylformamide, tetrahydrofuran and the like.

In method (b), the diester is first hydrolyzed under basic hydrolysis reaction conditions to provide the diacid 5, which is then converted to a compound of Formula (I) under the conditions described above. Syntheses of compounds of Formula (I), following the procedures described in Scheme B, are described in Examples 2-6.

Utility, Testing, and Administration

Utility

The present invention provides novel compounds possessing one or more of the following activities: antibacterial, antifungal and antitumor activity. The compounds and compositions containing them are therefore useful in the treatment of one or more of the following diseases: bacterial infections, fungal infections and cancer. Without wishing to be bound to any theory, Applicants believe that the antibacterial and antifungal activity of the compounds of Formula (I) is due to their binding to the minor groove of the double stranded DNA. Applicants further believe that the antitumor activity of the compounds of Formula (I) is due to their inhibition of topoisomerases.

Topoisomerases are essential enzymes in virtually all living cells. The enzymes have two distinct classes: type I and type II enzymes (J.C. Wang, review). Top I relaxes supercoiled DNA by transiently nicking one DNA strand and rotate one strand about the other. Top II relaxes supercoiled DNA and decatenate linked DNA by transiently cleaving both DNA strands and passing another DNA through the lesion. Since their discovery, topoisomerases have been widely targeted in cancer therapy.

Compounds of Formula (I) are also useful as ultraviolet (UV) light absorbers. Accordingly, they are suitable for use in compositions requiring a UV light absorbing additive, such as plastic compositions. In this regard, it is known that prolonged exposure to UV light can have a deleterious effect on the physical properties and compositional stability of certain plastics. It is therefore conventional to include a UV light absorbing additive in

such plastic compositions, and the compounds of Formula (I) can be employed in this manner.

Compounds of the present invention are further useful in that they bind to the minor groove of dsDNA thereby inducing DNA duplex formation. This property is beneficial in biological assays or diagnostic tests that measure the formation or stability of DNA duplexes. For instance, where one is attempting to measure the formation of a DNA duplex with a low T_m, one can increase the duplex population by adding a compound of Formula (I). Such an increase in population ensures that the binding event will be more easily measured. A compound of Formula (I) can also be used where one is detecting a single nucleotide polymorphism (SNP) through duplex formation. The compound will preferentially increase the T_m of a perfectly matched duplex over a single mutated duplex, therein allowing one to more easily distinguish the two.

Administration and Pharmaceutical Composition

In general, the compounds of this invention will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of this invention, i.e., the active ingredient, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors. The drug can be administered more than once a day, preferably once or twice a day.

Therapeutically effective amounts of compounds of Formula (I) may range from approximately 0.05 to 50 mg per kilogram body weight of the recipient per day; preferably about 0.01-25 mg/kg/day, more preferably from about 0.5 to 10 mg/kg/day. Thus, for administration to a 70 kg person, the dosage range would most preferably be about 35-70 mg per day.

In general, compounds of this invention will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (e.g., transdermal, intranasal or by suppository), or parenteral (e.g., intramuscular, intravenous or subcutaneous) administration. The preferred manner of administration is oral using a convenient daily dosage regimen which can be adjusted according to the degree of affliction. Compositions can take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate compositions.

Another preferred manner for administering compounds of this invention is inhalation. This is an effective method for delivering a therapeutic agent directly to the respiratory tract for the treatment of diseases such as asthma and similar or related respiratory tract disorders (see U. S. Patent 5,607,915).

The choice of formulation depends on various factors such as the mode of drug administration and bioavailability of the drug substance. For delivery via inhalation the compound can be formulated as liquid solution, suspensions, aerosol propellants or dry powder and loaded into a suitable dispenser for administration. There are several types of pharmaceutical inhalation devices-nebulizer inhalers, metered dose inhalers (MDI) and dry powder inhalers (DPI). Nebulizer devices produce a stream of high velocity air that causes the therapeutic agents (which are formulated in a liquid form) to spray as a mist which is carried into the patient's respiratory tract. MDI's typically are formulation packaged with a compressed gas. Upon actuation, the device discharges a measured amount of therapeutic agent by compressed gas, thus affording a reliable method of administering a set amount of agent. DPI dispenses therapeutic agents in the form of a free flowing powder that can be dispersed in the patient's inspiratory air-stream during breathing by the device. In order to achieve a free flowing powder, the therapeutic agent is formulated with an excipient such as lactose. A measured amount of the therapeutic agent is stored in a capsule form and is dispensed with each actuation.

Recently, pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area i.e., decreasing particle size. For example, U.S. Pat. No. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in which the active material is supported on a crosslinked matrix of macromolecules. U.S. Pat. No. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability.

The compositions are comprised of in general, a compound of Formula (I) in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the compound of Formula (I). Such excipient may be any solid, liquid, semi-solid or, in the case

of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

Compressed gases may be used to disperse a compound of this invention in aerosol form. Inert gases suitable for this purpose are nitrogen, carbon dioxide, etc.
Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

The amount of the compound in a formulation can vary within the full range employed by those skilled in the art. Typically, the formulation will contain, on a weight percent (wt%) basis, from about 0.01-99.99 wt% of a compound of Formula (I) based on the total formulation, with the balance being one or more suitable pharmaceutical excipients. Preferably, the compound is present at a level of about 1-80 wt%. Representative pharmaceutical formulations containing a compound of Formula (I) are described below.

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

The following abbreviations are employed: AcOEt for ethylacetate; DCE for 1,2-dichloroethane; DCM for dichloromethane; DIPEA for diisopropylethylamine; DMF for dimethylformamide; DMSO for dimethylsulfoxide; EtOH for ethanol; MeOH for methanol; THF for tetrahydrofuran; Pyr for pyridine; TFA for trifluoroacetic acid; DCC for N,N'-dicyclohexylcarbodiimide; DCU for N,N'-dicyclohexylurea; Me for a methyl radical; Et for an ethyl radical; Phe for a phenyl radical; Np for a 4-nitrophenyl radical; Pfp for a pentafluorophenyl radical; Gly for a glycine amino acid residue; Lys for a lysine amino acid residue; Arg for a arginine amino acid residue; Py for a 4-amino-1-methyl-1H-pyrrole-2-

carboxylic acid residue; Npc(Me) for a 4-nitro-1-methyl-1H-pyrrole-2-carboxylic acid residue; Npc(Et) for a 4-nitro-1-ethyl-1H-pyrrole-2-carboxylic acid residue; Npc(Pr) for a 4-nitro-1-propyl-1H-pyrrole-2-carboxylic acid residue; MMT for a monomethoxytrytil (p-anisyldiphenylmethyl) protecting group; Bzl for a benzyl protecting group; Boc for a tert-butoxycarbonyl protecting group; Fmoc for a fluorenylmethoxycarbonyl protecting group; Z for a benzyloxycarbonyl protecting group; t-Bu for a tert-butyl protecting group; Boc-5-Ain for N-Boc-5-Amino-Indole-2-Carboxylic Acid; Boc-5-Ain-HBA-AMPS for N-Boc-5-Amino-Indole-2-Carboxylic Acid (p-Hydroxy benzamide methyl polystyrene)ester; Boc Py for *N*-Boc-4-amino-1-methyl pyrrole-2-carboxylic acid; Boc-Py-HBA-AMPS for N-Boc-4-Amino-1-Methyl Pyrrole-2-Carboxylic Acid (p-Hydroxy benzamide methyl polystyrene)ester; BOP for Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate; DE for 2-(Dimethylamino)ethylamine; DIC for N,N' diisopropyl carbodiimide; DIEA for diisopropylethyl amine; DMAP for 4-Dimethylaminopyridine; DMF for dimethyl formamide; DP for 3-(Dimethylamino)propylamine; HBA-AMPS for p-hydroxybenzamide – methylpolystyrene; HBTU for O-Benzotriazol-1yl-N,N,N',N'-tetramethyluronium hexafluorophosphate; HCl for hydrochloric acid; Pzl-Gu-$(Boc)_2$ for *N,N'*-Bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine; TFA for Trifluoro acetic acid; NMR for nuclear magnetic resonance spectrum; MS for mass spectrum; TLC for thin layer chromatography on silica gel; HPLC for high pressure liquid chromatography; mp for melting point; mp d for melting point with decomposition. In reporting NMR data, chemical shifts are given in ppm and coupling constants (J) given in Hertz (Hz). All melting points are uncorrected.

Example 1

(Following scheme 1)

(A) Synthesis of indole-2,5-dicarboxylic acid, 1

Solution of 1H-Indole-2,5-dicarboxylic acid 2-ethyl ester[#] (20.0 g, 85.75 mmole) and NaOH (100 mmole) in a mixture of water/MeOH (1/1) (200 ml) was stirred at 50 °C for 4 h and then overnight at ambient temperature. The reaction mixture was evaporated *in vacuo* to dryness and the residue was dissolved in water (200 ml) and acidified with 1M HCl up to pH=3. The precipitates were collected on the filter and washed with water (3x50 ml) and dried over phosphorus pentoxide in dessicator to give indole-2,5-dicarboxylic acid 1 (10.38 g,

59%) of compound 1 as white crystals. MS: 203.7 (M-2H); 204.7 (M-H). ^{1}H-NMR (DMSO-d_6): 8.32 (m, 1H, H-4, indole); 7.80 (m, 1H, H-6, indole); 7.45 (d, 1H, H-7, indole); 7.22 (s, 1H, H-3, indole).

(B) 1H-Indole-2,5-dicarboxylic acid dipentafluorophenyl ester, 2

A solution of indole-2,5-dicarboxylic acid 1 (5.15 g, 25.1 mmole), pentafluorophenol (10.00 g, 52.7 mmole) and DCC (10.9 g, 52.7 mmole) in DMF (250 ml) was stirred for 16 h at ambient temperature and evaporated. The residue was coevaporated with toluene (3x100 ml) and recrystallized from the same solvent. Yield: 11.16 g (82.5%). ^{1}H-NMR (DMSO-d_6): 13.03 (s, 1H, H-1, indole); 8.76 (m, 1H, H-4, indole); 8.08 (m, 1H, H-6, indole); 7.84 (s, 1H, H-3, indole); 7.70 (d, 1H, H-7, indole). ^{19}F-NMR (DMSO-d_6): -153.22&-153.60 (m, 2F&2F, F-2&F-6, -OPfp); -157.15& -157.80 (m, 1F&1F, F-4, -OPfp); -162.03&-162.40 (m, 2F, F-3&F-5, -OPfp).

(C) Npc(Me)-OH, 3

A solution of Npc(Me)-OMe (18.4 g, 100.0 mmole) and NaOH (200 mmole) in a mixture of water/MeOH (2/3) (200 ml) was stirred at 50C for 6h and then overnight at ambient temperature and evaporated. The residue was dissolved in water (200 ml) and acidified with 1N HCl up to pH 2.0. The yellowish precipitate was collected, washed with water (5x250 ml) and dried *in vacuo* over phosphorus pentoxide to give Npc(Me)-OH, 3 16.16 g (95%) as a yellowish crystalline material. ^{1}H-NMR (DMSO-d_6): 7.61&7.40 (m&m, 1H&1H, H-3&H-5, Py); 3.87 (s, 3H, N$\underline{CH_3}$, Py).

(D) Npc(Me)-Cl, 4

Stirred suspension of Npc(Me)-OH 3 (13.66 g, 80.0 mmole) in thionyl chloride (50 ml) was gently refluxed 4 h and evaporated. The residue was coevaporated with dry toluene (3x50 ml) and used for the next step without purification.

(E) Npc(Me)-NHCH$_2$CH$_2$CN, 5

To a stirred solution of Npc(Me)-Cl 4 (80.0 mmole) and DIPEA (13.0 g, 17.4 ml, 100 mmole) in dry toluene (300 ml) at 0C aminopropionitrile (14.0 g, 200.0 mmole) was added dropwise. The reaction mixture was stirred at 0C for 30 min and at ambient temperature for 3h and evaporated. The residue was suspended in AcOEt (300ml) and washed with water

(2x100 ml), 0.1 M HCl (3x100 ml), brine (2x100 ml), 9.5% $NaHCO_3$ (3x100 ml) and brine (2x100 ml). The organic layer was dried over sodium sulfate and evaporated to give 17.70 g (99%) of Npc(Me)-$NHCH_2CH_2CN$ 5 as a white crystalline material. MS: 223.11 (M+H). ^{1}H-NMR (DMSO-d_6): 7.59&7.18 (m&m, 1H&1H, H-3&H-5, Py); 6.63 (t, 1H, -$\underline{NH}CH_2CH_2CN$); 3.99 (s, 3H, $N\underline{CH_3}$, Py); 3.67 (m, 2H, $NHC\underline{H_2}CH_2CN$); 2.74 (t, 2H, $NHCH_2C\underline{H_2}CN$).

(F) Npc(Me)-$NHCH_2CH_2C(=NH)NH_2$. HCl, 6

A suspension of Npc(Me)-$NHCH_2CH_2CN$ 5 (9.0 g, 40.5 mmole) in dry EtOH (250 ml) was saturated with HCl (gas) at ambient temperature and kept for 16 h at 0C and evaporated. The residue was co-evaporated with dry toluene (3x200 ml) and suspended in dry EtOH (250 ml). The suspension was saturated with ammonia (gas) at 0C and kept for 16 h at 0C and evaporated. The residue was crystallized from water-ethanol to give 8.26 g (74%) of Npc(Me)-$NHCH_2CH_2C(=NH)NH_2$ 6 as white crystalline material. ^{1}H-NMR (DMSO-d_6): 9.08&8.76 (bs&bs, 4H, $NHCH_2CH_2C(=NH)NH_2$. HCl); 8.13&7.52 (d&d, 1H&1H, H-3&H-5, Py); 3.99 (s, 3H, $N\underline{CH_3}$, Py); 3.51 (m, 2H, $NH\underline{CH_2}CH_2C(=NH)NH_2$); 2.62 (t, 2H, $NHCH_2\underline{CH_2}C(=NH)NH_2$).

(G) 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide 7

To a stirred solution of Npc(Me)-$NHCH_2CH_2C(=NH)NH_2$ 6 (55.2 mg, 0.20 mmole) in methanol (20 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigorously at 23°C for 1 hour. The suspended material was filtered off through a pad of Celite in a Buchner funnel and then the funnel was rinsed several times with a small portion of MeOH. The combined filtrate and washings was evaporated *in vacuo* to dryness. The resulted 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamidoyl-ethyl)-amide was used for the next step without purification.

A solution of compound 2 (51.0 mg, 0.095 mmole) and freshly prepared (as described above) 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamidoyl-ethyl)-amide (22.0 mmole) in dry DMF (2.0 ml) was kept at ambient temperature for 72 hours and evaporated. The residue was re-precipitated from MeOH-ether, the precipitate was dried *in vacuo* and dissolved in water (5.0 ml). The resulted water solution was filtered through 0.45 μm filter and lyophilized to give 53 mg (83%) of 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-

carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide 7. MS: 294.60 (doubly charged peak, (M+H)/2). ^{1}H-NMR (DMSO-d_6):11.97 (s, 1H, H-1, indole); 10.54&10.22 (s&s, 1H&1H, -C(=O)NH); 9.01&8.67 (bs&bs, 4H&4H, $NHCH_2CH_2C(=NH)NH_2$ x HCl); 8.31 (m, 2H with *, H-6, indole); 8.30 (m, *, $NHCH_2CH_2C(=NH)NH_2$ x HCl); 8.25 (t, 1H, $NHCH_2CH_2C(=NH)NH_2$ x HCl); 7.79 (m, 1H, H-6, indole); 7.48 (m, 1H, H-7, indole); 7.45 (s, 1H, H-3, indole); 7.31&7.28&6.97&6.96 (s&s&d&s, 4H, H-3&H-5, Py_1&Py_2); 3.83&3.81 (s&s, 6H, NCH_3, Py_1&Py_2); 3.50 (m, 4H, $NHCH_2CH_2C(=NH)NH_2$); 2.62 (m, 4H, $NHCH_2CH_2C(=NH)NH_2$).

Example 2

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[5-(2-amino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}

(A) 4-({1-[2-(5-methoxycarbonyl-1methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid methyl ester, 8

To a stirred solution of compound methyl 4-nitro-1-methyl-1H-pyrrole-2-carboxylate (967 mg, 5.25 mmole) in a mixture of AcOEt/EtOH (3/2) (50 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.2g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigrously at 23°C for 1 hour. The suspended material was filtered off through a pad of Celite in a Buchner funnel and then the funnel was rinsed several times with a small portion of AcOEt and EtOH. The combined filtrate and washings was evaporated *in vacuo* to dryness. The resulted methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate was used for the next step without purification.

A solution of compound 7 (1.13, 2.1 mmole) and freshly prepared (as described above) methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate in dry DMF (10.0 ml) was kept at ambient temperature for 48 hours and evaporated. The residue was re-precipitated from DMF (10 ml)-0.01 M HCl (100 ml). The precipitate was collected on the filter, washed with water (3x5 ml) end ether (2x3 ml) and dried *in vacuo* over phosphorus pentoxide to give 4-({1-[2-(5-methoxycarbonyl-1methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid methyl ester 8 with quantitative yield. MS: 478.14 (M+H). ^{1}H-NMR (DMSO-d_6): 11.96 (s, 1H, H-1, indole); 10.43&10.23 (s&s, 1H&1H, -C(=O)NH); 8.29 (m, 1H, H-6, indole); 7.50 (m, 3H, H-7, indole; H-3 or H-5, Py_1&Py_2); 7.37

(s, 1H, H-3, indole); 6.95 (m, 2H, H-3 or H-5, Py_1&Py_2); 3.86&3.85 (s, 6H, N$\underline{CH_3}$, Py_1&Py_2); 3.74&3.73 (s, 6H, O$\underline{CH_3}$, Py_1&Py_2).

(B) 4-({1-[2-(5-hydroxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2-carboxylic acid, 9

A solution of 4-({1-[2-(5-methoxycarbonyl-1methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid methyl ester 8 (1.04 g, 2.18 mmole) and NaOH (10 mmole) in a mixture of water/MeOH (1/4) (25 ml) was stirred at 50C for 6h and then overnight at ambient temperature. The reaction mixture was evaporated *in vacuo* to dryness and the residue was dissolved in water (50 ml) and acidified with 1M HCl up to pH=3. The precipitate was collected on the filter and washed with water (3x50 ml) and dried over phosphorus pentoxide in dessicator to give 0.85 g (87%) of 4-({1-[2-(5-hydroxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2-carboxylic acid 9 as white crystalline material. MS: 448.08 (M-H). ^{1}H-NMR (DMSO-d_6): 11.96 (s, 1H, H-1, indole); 10.45&10.25 (s&s, 1H&1H, -C(=O)$\underline{NH}$); 8.30 (m, 1H, H-6, indole); 7.48 (m, 3H with *, H-7, indole); 7.49&7.46 (d&d, *, H-3 or H-5, Py_1&Py_2); 7.39 (s, 1H, H-3, indole); 6.91 (m, 2H, H-3 or H-5, Py_1&Py_2); 3.84&3.83 (s&s, 6H, N$\underline{CH_3}$, Py_1&Py_2).

(C) 4-({1-[2-(5-pentafluorophenoxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid pentafluorophenyl ester, 10

A solution of 4-({1-[2-(5-hydroxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2-carboxylic acid 9 (0.85 g, 1.86 mmole), pentafluorophenol (0.72 g, 3.9 mmole) and DCC (0.81 g, 3.9 mmole) in DMF (15 ml) was stirred for 16 h at ambient temperature and evaporated. The residue was coevaporated with toluene (3x100 ml) and chromatographed over a silica gel column (2.5x25 cm) using mixture of toluene/AcOEt (7:3), as eluent to give 1.23 g (84%) of 4-({1-[2-(5-pentafluorophenoxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid pentafluorophenyl ester 10. ^{1}H-NMR (DMSO-d_6): 12.03 (s, 1H, H-1, indole); 10.60&10.41 (s&s, 1H&1H, -C(=O)$\underline{NH}$); 7.81 (m, 3H with *, H-6, indole); 7.80&7.78 (d&d, *, H-3 or H-5, Py_1&Py_2); 7.52 (m, 1H, H-6, indole); 7.41 (s, 1H, H-3, indole); 7.34 (m, 2H, H-3 or H-5, Py_1&Py_2); 3.92&3.90 (s&s, 6H, N$\underline{CH_3}$, Py_1&Py_2). ^{19}F-NMR (DMSO-d_6): -153.56&-153.60 (m&m, 2F&2F, F-2&F-6, -

OPfp); -158.17&-158.27 (m&m, 1F&1F, F-4, -OPfp); -162.69 & -162.73 (m&m, 2F&2F, F-3&F-5, -OPfp).

(D) 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-amino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}11

A solution of of 4-({1-[2-(5-pentafluorophenoxycarbonyl-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1-methyl-1H-pyrrole-2carboxylic acid pentafluorophenyl ester 10 (150 mg, 0.192 mmole) and ethylenediamine-1,2 (0.26 ml, 3.94 mmole)in dry DMF (2.0 ml) was kept at ambient temperature for 24 hours and evaporated. The residue was dissolved in 0.1 TFA and purified by HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min) to give 1H-indole-2,5-dicarboxylic acid bis-{[5-(2-amino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide} 11, as a bis-trifluoroacetate salt: 56 mg (38%). ES MS: 534.28 (calcd. for $M+H^+$: 534.28).

Example 3

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-dimethylamino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 12

Compound 12 was synthesized as described for Compound 11 above. Yield: (35%) of compound 12. ES MS: 590.32 (calcd. for $M+H^+$: 590.32).

Example 4

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-amino-propylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 13

Compound 13 was synthesized as described for Compound 11 above. Yield: (37%) of compound 12. The structure was confirmed by ES MS.

Example 5

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-dimethylamino-propylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 14

Compound 12 was synthesized as described for Compound 11 above. Yield: (31%) of compound 12. The structure was confirmed by ES MS.

Example 6

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[1-methyl-5-(2-piperazin-1-yl-ethylcarbamoyl)-1H-pyrrol-3-yl]-amide}, 15

Compound 15 was synthesized as described for Compound 10 above. Yield: (15%) of compound 15. The structure was confirmed by ES MS.

Example 7

Synthesis of 1-methyl-indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 20

(A) 1H-Indole-2,5-dicarboxylic acid diethyl ester, 16

A suspension of 1H-Indole-2,5-dicarboxylic acid 2-ethyl ester (20.0 g, 85.75 mmole) in a saturated HCl/ EtOH (200 ml) was stirred at 55C for 24h and evaporated *in vacuo* to dryness. The residue was freeze-dried from dioxane to give 22.18 g (99%) of 1H-indole-2,5-dicarboxylic acid diethyl ester, 16 as white powder. MS: 262.12 (M+H). ^{1}H-NMR (DMSO-d_6): 12.23 (s, 1H, H-1, indole); 8.34 (m, 1H, H-4, indole); 7.83 (m, 1H, H-6, indole); 7.49 (m, 1H, H-7, indole); 7.30 (s, 1H, H-3, indole); 4.31 (m, 4H, -O$\underline{CH_2}CH_3$); 1.32 (m, 4H, -O$CH_2\underline{CH_3}$).

(B) 1-Methyl-indole-2,5-dicarboxylic acid diethyl ester, 17

Sodium hydride (60%-suspension, 144 mg, 3.6 mmole) was added to a stirred solution of 1H-indole-2,5-dicarboxylic acid diethyl ester 16 (784 mg, 3.0 mmole) in dry DMF (15.0 ml) and kept at ambient temperature for 30 min. To a resulted reaction mixture MeI (280 µl, 4.5 mmol) was added and kept at ambient temperature for 16 hours and evaporated. The residue was suspended in AcOEt (100ml) and washed with water (2x20 ml), 0.01 M HCl (3x20 ml) and brine (2x100 ml). The organic layer was dried over sodium sulfate and evaporated to give 800 mg (97%) of 1-methyl-indole-2,5-dicarboxylic acid diethyl ester 17 as a yellow oil. ^{1}H-NMR (DMSO-d_6): 8.45 (m, 1H, H-4, indole); 8.03 (m, 1H, H-6, indole);

7.40 (m, 1H, H-7, indole); 7.38 (s, 1H, H-3, indole); 4.40 (m, 4H, -O$\underline{CH_2}CH_3$); 4.10 (s, 3H, N$\underline{CH_3}$, indole); 1.42 (m, 4H, -$OCH_2\underline{CH_3}$).

(C) 1-Methyl-indole-2,5-dicarboxylic acid, 18

Solution of 1-methyl-indole-2,5-dicarboxylic acid diethyl ester 17 (675 mg, 2.5 mmole) and NaOH (5.00 mmole) in a mixture of water/MeOH (1/4) (20 ml) was stirred at 50C for 4h and then overnight at ambient temperature. The reaction mixture was evaporated *in vacuo* to dryness and the residue was dissolved in water (20 ml) and acidified with 1M HCl up to pH=3. The precipitate was collected on the filter and washed with water (3x5 ml) and dried over phosphorus pentoxide in dessicator to give 488 mg (89%) of 1-methyl-indole-2,5-dicarboxylic acid 18 as white crystalline material. MS: 218.2 (M-H). ^{1}H-NMR (DMSO-d_6): 8.35 (m, 1H, H-4, indole); 7.90 (m, 1H, H-6, indole); 7.41 (d, 1H, H-7, indole); 7.32 (s, 1H, H-3, indole); 4.05 (s, 3H, N$\underline{CH_3}$, indole).

(D) 1-Methyl-indole-2,5-dicarboxylic acid di(2,3,5,6-tetrafluorophenyl) ester, 19

To a stirred solution of 1-methyl-indole-2,5-dicarboxylic acid 18 (439 mg, 2.0 mmole), triethylamine (12 mmole) in dry DCM (20 ml), maintained at 0C, the solution of tetrafluorophenyl trifluoroacetate (6 mmole) in dry DCM (20 ml) was added dropwise. The stirred reaction mixture was kept on ice bath for 2 h and then overnight at ambient temperature. The reaction mixture was evaporated *in vacuo* to dryness and the residue was dissolved in DMF (20 ml) and the solution used as it was for the next step reaction.

(E) 1-Methyl-indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 20

To a stirred solution of compound 6 (110 mg, 0.40 mmole) in methanol (20 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigrously at 23°C for 1 hour. The suspended material was filtered off through a pad of Celite in a Buchner funnel and then the funnel was rinsed several times with a small portion of MeOH. The combined filtrate and washings was evaporated *in vacuo* to dryness. The resulted 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamidoyl-ethyl)-amide was used for the next step without purification.

A solution of 1-methyl-indole-2,5-dicarboxylic acid di(2,3,5,6-tetrafluorophenyl) ester 19 (2.0 ml, 0.2 mmole; see Example 19) and freshly prepared (as described above) 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamidoyl-ethyl)-amide (0.4 mmole) in dry DMF (2.0 ml) was kept at ambient temperature for 72 hours and evaporated. The residue was re-precipitated from MeOH-ether, the precipitate was dried *in vacuo*, dissolved in 0.1 TFA and purified by HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min) to give 1-methyl-indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide} 20, as a bis-trifluoroacetate salt: mg (18%). The structure was confirmed by ES MS.

Example 8

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-1H-indol-5-yl]-amide}

(A) 5-({1-[2-(2-ethoxycarbonyl-1H-indol-5-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1H-indole-2-carboxylic acid ethyl ester, 21

To a stirred solution of 5-nitroindole-2-carboxylic acid ethyl ester (220 mg, 0.93 mmole) in methanol (10 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigrously at 23°C for 1 hour. The suspended material was filtered off through a pad of Celite in a Buchner funnel and then the funnel was rinsed several times with a small portion of MeOH. The combined filtrate and washings was evaporated *in vacuo* to dryness. The resulted 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamidoyl-ethyl)-amide was used for the next step without purification.

A solution of compound 2 (200 mg, 0.372 mmole) and freshly prepared of 5-aminoindole-2-carboxylic acid ethyl ester (as described above) methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate in dry DMF (5.0 ml) was kept at ambient temperature for 48 hours and evaporated. The residue was re-precipitated from DMF (1.0 ml)-0.01 M HCl (10 ml). The precipitate was collected on the filter, washed with water (3x5 ml) end ether (2x3 ml) and dried *in vacuo* over phosphorus pentoxide to give 142 mg (66%) of 5-({1-[2-(2-ethoxycarbonyl-1H-indol-5-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1H-indole-2-carboxylic acid ethyl ester 21. The structure was confirmed by ES MS and ^{1}H-NMR.

(B) 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-1H-indol-5-yl]-amide}, 22

A solution of 5-({1-[2-(2-ethoxycarbonyl-1H-indol-5-ylcarbamoyl)-1H-indol-5-yl]-methanoyl}-amino)-1H-indole-2-carboxylic acid ethyl ester 21 (115 mg, 0.2 mmole) and ethylenediamine-1,2 (1.5 ml) in dry DMF (2.0 ml) was kept at 55C for 16 h and evaporated. The residue was dissolved in 0.1 TFA and purified by HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min) to give 1H-indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-1H-indol-5-yl]-amide} 22, as a bis-trifluoroacetate salt: 62 mg (%). ES MS: 606.27 ($M+H^+$).

Example 9

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-guanidino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}

(A) MMT-$NHCH_2CH_2NH_2$, 23

MMT-Cl (15.44 g, 50 mmole) was added dropwise to a stirred solution of ethylenediamine (24.0 g, 400 mmole) in DCM (500 ml) at 0C. The reaction mixture was kept at ambient temperature for 2 h, washed with $NaHCO_3$ (5 x 100 ml) and water (3 x 100 ml), dried over sodium sulfate and evaporated. The residue was chromatographed over a silica gel column (5.0x25 cm) using mixture of chloroform/MeOH (19:1+0.01% of ammonia), as eluent to give 11.38 g (68%) of MMT-$NHCH_2CH_2NH_2$ 23 as light yellow foam. The structure was confirmed by ^{1}H-NMR.

(B) MMT-$NHCH_2CH_2NHC$(=N-Boc)NH-Boc, 24

A solution of MMT-$NHCH_2CH_2NH_2$ 23 (8.06 g, 24.24 mmole), 1-H-pyrazole-1-[N,N'-bis(tert-butoxycarbonyl)carboxamidine (6.02 g, 19.4 mmole) in MeCN (100 ml) was stirred for 16 h at ambient temperature and evaporated. The residue was coevaporated with toluene (3x100 ml) and chromatographed over a silica gel column (2.5x25 cm) using mixture of hexane/AcOEt (9:1), as eluent to give 10.71 g (96%) of MMT-$NHCH_2CH_2NHC$(=N-Boc)NH-Boc 24. The structure was confirmed by ES MS and ^{1}H-NMR.

(C) $NH_2CH_2CH_2NHC(=N\text{-}Boc)NH\text{-}Boc$, 25

To a stirred solution of MMT-$NHCH_2CH_2NHC(=N\text{-}Boc)NH\text{-}Boc$ 24 (7.0 mg, 12.2 mmole) in the mixture AcOEt/MeOH (3:1, 200 ml) was added 10% Pd/C (Degussa type, Aldrich) (1.0 g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 40 to 50 psi. The resultant suspension was stirred vigrously at 23°C for 24 hour. The suspended material was filtered off through a pad of Celite in a Buchner funnel and then the funnel was rinsed several times with a small portion of MeOH. The combined filtrate and washings was evaporated *in vacuo* to dryness. The resulted compound $NH_2CH_2CH_2NHC(=N\text{-}Boc)NH\text{-}Boc$ 25 was used for the next step without purification.

(D) 1H-Indole-2,5-dicarboxylic acid bis-{[5-(2-guanidino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 26

A solution of compound 10 (78.2 mg, 0.10 mmole) and $NH_2CH_2CH_2NHC(=N\text{-}Boc)NH$-B 25 (144 mg, 0.25 mmole)in dry DMF (1.0 ml) was kept at ambient temperature for 24 hours and evaporated. The residue was dissolved in the mixture TFA/DCM/anisole (49/49/2), kept at ambient temperature for 1 h and evaporated. The residue was dissolved in 0.1% TFA purified by HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min) to give 1H-indole-2,5-dicarboxylic acid bis-{[5-(2-guanidino-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}, 26, as a bis-trifluoroacetate salt: 56 mg (38%). ES MS: 618.32 (M+H).

EXAMPLE 10

Synthesis of 1H-pyrrole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-amide} **30**

(A) Pyrrole-2,5-dicarboxylic acid (**31**)

Pyrrole-2,5-dicarbaldehyde was prepared in three steps according to the literature (R. Miller and K. Olsson, *Acta Chemica Scandinavica* B, 1981, *35*, 303-304) from pyrrole-2-carboxaldehyde. ^1H NMR (DMSO-d_6) δ 13.08 (br. S), 9.74 (s), 7.04 (s).

Pyrrole-2,5-dicarbaldehyde (0.21 g, 1.71 mmol) was dissolved in 35 ml of hot water and placed in a hot water bath (95-100 °C). A solution of $KMnO_4$ (0.788 g, 5.13 mmol) in 10 ml of water was added dropwise in a period of 5 min. The reaction mixture was stirred at 95-100 °C for 1 h, and was then cooled to 70 °C. The brown precipitates (MnO_2) were filtered

off and washed with water. The filtrate was acidified at 0 °C with 5 M HCl to pH 2, evaporated to dryness, and dried under high vacuum. The product was dissolved in 80 ml of anhydrous EtOH and the solution was filtered through a funnel. The filtrate was evaporated to give a brown solid (0.25 g), which was used in next reaction without further purification. ESI MS: 154.00 ($M - H^+$). ^1H NMR (DMSO-d_6) δ 12.68 (br. S), 12.17 (s), 6.72 (s).

(B) Pyrrole-2,5-dicarboxylic acid dipentafluorophenyl ester (**32**)

To a solution of pyrrole-2,5-dicarboxylic acid (0.24 g) in 10ml of anhydrous DMF in the presence of triethylamine (0.48 ml, 3.42 mmol) was added dropwise pentafluorophenyl trifluoroacetate in 2 min at 0 oC. The reaction mixture was warmed up slowly to room temperature and stirred at room temperature overnight. After evaporation of solvent, the residue was dissolved in 30 ml of ethyl acetate, washed with water 30 ml X 3) and dried over Na_2SO_4. The solvent was evaporated and the product was adsorbed onto silica gel, which was placed on the top of silica gel column to run the flash chromatography by using toluene-ethyl acetate (30:1) as eluent. The product as small crystals were obtained (0.406 g). ^{19}F NMR ($CDCl_3$) δ -152.13 (d), -156.80 (t), -161.60 (t). The total yield for the above two steps reaction was 49%.

(C) 1H-Pyrrole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-amide} (**33**)

General Procedure A: To a solution of 4-nitro-1-propyl-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide (52 mg, 0.2 mmol) in 15 ml of MeOH was added 20 mg of 5% Pd/C under argon. The reaction mixture was flashed with hydrogen and shaken under hydrogen at 30 psi for 30 min. The catalyst was removed by filtration through celite and washed with methanol. The filtrate was evaporated to dryness to give 4-amino-1-propyl-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide **34**. This product was immediately used in next step reaction.

General Procedure B: A mixture of above amine **34** and pyrrole-2,5-dicarboxylic acid dipentafluorophenyl ester **32** (34.1 mg, 0.07 mmol) in 2 ml of anhydrous DMF under argon was stirred at 55 °C overnight. The product was directly purified by reverse phase HPLC (Zorbax SB-C18 2.2X25 cm; Mobile phase: A = water with 0.1% TFA, B = CH_3CN with 0.1% TFA; Gradient: 0 to 60% B, 40 min; Flow rate: 10 ml/min). The purified compound was transferred to its HCl salt by dissolving it in 2 ml of methanol, following addition 0.5 ml

of saturated ethanol with HCl gas or 4 N HCl in dioxane at 0 °C. The solution was diluted with 40 ml of cold anhydrous ether and the precipitates were collected and dried. The total yield was 21.2 mg (45%). ESI MS: 594.32 ($M + H^+$). 297.66 ($M/2 + H^+$).

EXAMPLE 11

Synthesis of 1H-pyrrole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **35**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide **36** (60 mg, 0.2 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide **37** by hydrogenation according to general procedure A in example 10.

Pyrrole-2,5-dicarboxylic acid dipentafluorophenyl ester **32** (34.1 mg, 0.07 mmol) was condensed with above amine according to general procedure B in example 10 to give **35** (24.2 mg, 48%). ESI MS: m/z 650.39 ($M + H^+$), 325.69 ($M/2 + H^+$).

EXAMPLE 12

Synthesis of 1H-pyrrole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide} **38**

1-Methyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide **39** (47.8 mg, 0.2 mmol) was reduced to 4-amino-1-methyl-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide **40** by hydrogenation according to general procedure A in example 10.

Pyrrole-2,5-dicarboxylic acid dipentafluorophenyl ester **32** (34.1 mg, 0.07 mmol) was condensed with above amine according to general procedure B in example 10 to give **38** (31.5 mg, 74%). ESI MS: 538.26 ($M + H^+$) 269.63 ($M/2 + H^+$).

EXAMPLE 13

Synthesis of thiophene-2,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **41**

(A) N-(3-Cyanopropyl)phthalimide (**42**)

A mixture of potassium phthalimide (8.48 g, 0.046 mol) and 4-bromopropylcyanide (6.4 g, 0.043 mol) in 50 ml of anhydrous DMF was stirred at 90 °C for 2 h. After dilution with 300 ml of water, the aqueous solution was extracted with chloroform (80 ml X 3). The combined chloroform solution was washed with 0.5% NaOH aqueous solution (80 ml) and water (100 ml), and dried over anhydrous Na2SO4. After evaporation of chloroform, an oil was obtained and 300 ml of water was added. The oil was rapidly solidified. The solid formed was collected by filtration, washed with water, and dried under high vacuum. The product was recrystallized from methanol which was diluted with water, to give white crystals (7.75 g, 84%). ^{1}H NMR ($CDCl_3$) δ 7.87 (dd, 2H), 7.75 (dd, 2H), 3.83 (t, 2H), 2.44 (t, 2H), 2.09 (quintet, 2H).

(B) 3-Amino-propylcyanide hydrochloride (**43**)

A mixture of N-(3-cyanopropyl)phthalimide (7.56 g, 35.29 mmol) and hydrazine hydrate (4.4 g, 88.22 mmol) in 20 ml of ethanol was stood at room temperature overnight. After the solution was diluted with 8 ml of water, it was adjusted to pH 3.5 with hydrochloric acid and the precipitates were removed by filtration. The filtrate was evaporated to a small volume. The residue was cooled to 0 °C and then treated with 10 N NaOH solution (6 ml). This basic solution was extracted with chloroform (80 ml X 4). The combined chloroform solution was dried over Na_2SO_4 and evaporated to dryness. The residue was extracted with ether (100 ml) and precipitated after anhydrous HCl was passed through ether solution. A white solid was obtained (2.2 g, 51%). ^{1}H NMR (DMSO-d_6) δ 8.12 (br, s), 2.82 (m, 2H), 2.61 (t, 2H), 1.86 (quintet, 2H).

(C) 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester (**44**)

4-Nitro-1H-pyrrole-2-carboxylic acid ethyl ester (3.69 g, 20.04 mmol) was dissolved in 100 ml of hot anhydrous ethanol and the solution was cooled to room temperature. 30 ml of sodium ethoxide (about 1M) in ethanol was added. The reaction mixture was stirred at room temperature for 20 min and 1-bromo-3-methylbutane (8 ml) was added. The mixture was stirred at reflux for 6 h and cooled to room temperature and then poured into water. The pale yellow precipitates were collected by filtration, washed with water, and dried to give the product (1.48 g, 29%).

(D) 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carbonyl chloride (**45**)

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester (1.44 g,) was dissolved in 50 ml of methanol and 25 ml of 20% aqueous NaOH was added. The reaction mixture was stirred at 50 °C for 1.5 h until there was no starting material checked by TLC. The reaction mixture was concentrated to about 20 ml, 200 ml of water was added. The resulting solution was neutralized with 5 M hydrochloric acid to pH 2 and the precipitates formed were collected by filtration, washed with water and dried to give 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (1.29 g, 99%). ^{1}H NMR (DMSO-d_6) δ 13.12 (br. s, 1H), 8.27 (d, 1H), 7.26 (d, 1H), 4.36 (t, 2H), 1.59 (dd, 2H), 1.50 (dt, 1H), 0.88 (d, 6H).

The acid was suspended in 15 ml of $SOCl_2$. The reaction mixture was stirred at reflux under argon for 4 h, cooled to room temperature, and evaporated. To the residue was added 80 ml of anhydrous toluene and the toluene evaporated. This was repeated three times. The residue was dissolved in 20 ml of anhydrous benzene, which was frozen and lyophilized to give a white powder (1.26 g, 99%).

(E) 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-cyano-propyl)-amide (**46**)

To a mixture of the acid chloride **45** (0.6 g, 2.45 mmol) and 3-aminopropylcyanide hydrochloride (0.31 g, 2.57 mmol) in anhydrous toluene was added 1.5 ml of anhydrous pyridine. The reaction mixture was stirred at 50 °C overnight and then evaporated to dryness. The product was purified by chromatography using toluene-ethyl acetate (2:1) to yield white crystals (0.623 g, 87%). ^{1}H NMR (DMSO-d_6) δ 8.46 (t, 1H), 8.17 (d, 1H), 7.39 (d, 1H), 4.37 (t, 2H), 3.27 (quintet, 2H), 2.53 (d, 2H), 1.77 (quintet, 2H), 1.60-1.42 (q and quintet, 3H), 0.87 (d, 6H).

(F) 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**)

48 (0.6 g, 2.05 mmol) was dissolved in 25 ml of anhydrous ethanol and the solution was cooled to 0 °C in an ice-bath. Then hydrogen chloride gas dried through concentrated H_2SO_4 was bubbled through the solution for 1.5 h. The reaction flask was stopped by using a rubber stopper. The above saturated solution was stirred at room temperature for 4 h and was placed in a refrigerator overnight. Evaporation of solvent gave an oil. To the residue was added 80 ml of anhydrous toluene and the toluene evaporated. This was repeated twice. The white solid obtained was dried under high vacuum.

The product was dissolved in 40 ml of anhydrous ethanol and anhydrous ammonia gas was bubbled through the solution at room temperature for 1.5 h. The flask was stopped by using a rubber stopper. The reaction solution was stirred at 50 °C for 1 h and left at room temperature overnight. The solvent was evaporated and co-evaporated with anhydrous toluene twice. The residue was dried under high vacuum, then dissolved in 1 ml of anhydrous methanol and the solution was diluted with 45 ml of cold anhydrous ether. The precipitates were collected by centrifuge and dried to give a white powder (0.63 g, 89%). ESI MS: 310.19 ($M + H^+$). ^1H NMR (DMSO-d_6) δ 8.97 (br. s, 2H), 8.56 (br, s, 3H), 8.18 (d, 1H), 7.44 (d, 1H), 4.37 (t, 2H), 3.21 (q, 2H), 2.40 (t, 2H), 1.80 (quintet, 2H), 1.56 (quintet, 2H), 1.46 (quintet, 1H), 0.87 (d, 6H).

(G) Thiophene-2,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} (**49**)

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (48.4 mg, 0.14 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **50** by hydrogenation according to general procedure A in example 10.

5-Pentafluorophenylcarbamoyl-thiophene-2-carboxylic acid pentafluorophenyl ester **50** (25.2 mg, 0.05 mmol) was condensed with above amine **51** according to general procedure B in example 10 to give GL800918 (28.7 mg, 75%). ESI MS: 695.35 ($M + H^+$), 348.18 ($M/2 + H^+$).

EXAMPLE 14

Synthesis of thiophene-2,5-dicarboxylic acid bis-{[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **52**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (50.4 mg, 0.14 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

5-Pentafluorophenylcarbamoyl-thiophene-2-carboxylic acid pentafluorophenyl ester **50** (25.2 mg, 0.05 mmol) was condensed with above amine **54** according to general procedure B to give **52** (28.3 mg, 71%). ESI MS: 723.39 ($M + H^+$), 362.20 ($M/2 + H^+$).

EXAMPLE 15

Synthesis of 1H-pyrazole-3,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **55**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (17.3 mg, 0.05 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

5-Pentafluorophenylcarbamoyl-2H-pyrazole-3-carboxylic acid pentafluorophenyl ester **56** (8.8 mg, 0.018 mmol) was condensed with above amine **51** according to general procedure B in example 10 to give **55** (5.6 mg, 41%). ESI MS: 679.43 ($M + H^+$), 340.22 ($M/2 + H^+$).

EXAMPLE 16

Synthesis of 1H-pyrazole-3,5-dicarboxylic acid bis-{[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **57**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (18 mg, 0.05 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

5-Pentafluorophenylcarbamoyl-2H-pyrazole-3-carboxylic acid pentafluorophenyl ester **56** (8.8 mg, 0.018 mmol) was condensed with above amine **54** according to general procedure B in example 10 to give GL190472 (5.2 mg, 37%). ESI MS: 707.46 ($M + H^+$), 354.24 ($M/2 + H^+$).

EXAMPLE 17

Synthesis of N,N'-Bis-[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **58**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride **47** (50 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-

pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

Terephthalic acid dipentafluorophenyl ester **59** (30 mg, 0.06 mmol) was condensed with above amine **51** according to general procedure B in example 10 to give GL324265 (9.4 mg, 21%). ESI MS: 689.40 ($M + H^+$), 345.21 ($M/2 + H^+$).

EXAMPLE 18

Synthesis of N,N'-bis-[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **60**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (54 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

Terephthalic acid dipentafluorophenyl ester **59** (30 mg, 0.06 mmol) was condensed with above amine **54** according to general procedure B in example 10 to give **60** (29.9 mg, 63%). ESI MS: 359.23 ($M/2 + H^+$).

EXAMPLE 19

Synthesis of pyridine-2,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **61**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (48.4 mg, 0.14 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

Pyridine-2,5-dicarboxylic acid dipentafluorophenyl ester **62** (25 mg, 0.05 mmol) was condensed with above amine according to general procedure B in example 10 to give **61** (35.7 mg, 89%). ESI MS: 345.74 ($M/2 + H^+$).

EXAMPLE 20

Synthesis of pyridine-2,5-dicarboxylic acid bis-{[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **63**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (54 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

Pyridine-2,5-dicarboxylic acid dipentafluorophenyl ester **62** (30 mg, 0.06 mmol) was condensed with above amine **54** according to general procedure B in example 10 to give **63** (28.5 mg, 57%). ESI MS: 718.46 ($M + H^+$), 359.73 ($M/2 + H^+$).

EXAMPLE 21

Synthesis of pyrazine-2,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **64**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (52 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

Pyrazine-2,5-dicarboxylic acid dipentafluorophenyl ester **65** (30 mg, 0.06 mmol) was condensed with above amine **51** according to general procedure B in the example 10 to give **64** (30 mg, 65%). ESI MS: 691.40 ($M + H^+$), 346.20 ($M/2 + H^+$).

EXAMPLE 22

Synthesis of pyrazine-2,5-dicarboxylic acid bis-{[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **66**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (54 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

Pyrazine-2,5-dicarboxylic acid dipentafluorophenyl ester **65** (30 mg, 0.06 mmol) was condensed with above amine according to general procedure B in example 10 to give **66** (29 mg, 61%). ESI MS: 360.22 ($M/2 + H^+$).

EXAMPLE 23

Synthesis of N^1,N^4-bis-[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-2-methyl-terephthalamide **67**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (52 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

2-Methyl-terephthalic acid dipentafluorophenyl ester **68** (30.7 mg, 0.06 mmol) was condensed with above amine DS-c-2 according to general procedure B to give **67** (20.5 mg, 44%). ESI MS: 352.22 ($M/2 + H^+$).

EXAMPLE 24

Synthesis of N^1,N^4-bis-[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-2-methyl-terephthalamide **69**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (54 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

2-Methyl-terephthalic acid dipentafluorophenyl ester **68** (30.7 mg, 0.06 mmol) was condensed with above amine **54** according to general procedure B in example 10 to give **69** (25 mg, 52%). ESI MS: 366.23 ($M/2 + H^+$).

EXAMPLE 25

Synthesis of N,N'-bis-[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-2,5-dimethyl-terephthalamide **70**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (52 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

2,5-Dimethyl-terephthalic acid dipentafluorophenyl ester **71** (31.6 mg, 0.06 mmol) was condensed with above amine according to general procedure B in example 10 to give **70** (19.1 mg, 40%). ESI MS: 359.22 ($M/2 + H^+$).

EXAMPLE 26

Synthesis of N,N'-bis-[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-2,5-dimethyl-terephthalamide **72**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (54 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

2,5-Dimethyl-terephthalic acid dipentafluorophenyl ester **71** (31.6 mg, 0.06 mmol) was condensed with above amine **54** according to general procedure B in example 10 to give **72** (22.8 mg, 46%). ESI MS: 373.24 ($M/2 + H^+$).

EXAMPLE 27

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[5-(3-carbamimidoyl-propylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **73**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide hydrochloride (**47**) (48.4 mg, 0.14 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (3-carbamimidoyl-propyl)-amide **51** by hydrogenation according to general procedure A in example 10.

1H-Indole-2,5-dicarboxylic acid bis-(pentafluorophenyl-amide) **74** (26.8 mg, 0.05 mmol) was condensed with above amine **51** according to general procedure B in example 10 to give **73** (29.6 mg, 74%). ESI MS: 728.42 ($M + H^+$), 364.71 ($M/2 + H^+$).

EXAMPLE 28

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[5-(4-carbamimidoyl-butylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **75**

1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide hydrochloride **53** (50.4 mg, 0.15 mmol) was reduced to 4-amino-1-(3-methyl-butyl)-1H-pyrrole-2-carboxylic acid (5-carbamimidoyl-pentyl)-amide **54** by hydrogenation according to general procedure A in example 10.

1H-Indole-2,5-dicarboxylic acid bis-(pentafluorophenyl-amide) **74** (26.8 mg, 0.05 mmol) was condensed with above amine according to general procedure B in example 10 to give **75** (13 mg, 31%). ESI MS: 756.44 ($M + H^+$), 378.73 ($M/2 + H^+$).

EXAMPLE 29

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[2-(2-ethylamino-ethylcarbamoyl)-1H-indol-5-yl]-amide} **76**

(A) 5-Nitro-1H-indole-2-carboxylic acid (2-ethylamino-ethyl)-amide (**77**)

A fine powder of 5-nitro-2-indolecarboxylic acid ethyl ester (0.8 g, 3.41 mmol) was suspended in 2 ml of N-ethylethylenediamine under argon and the reaction mixture was stood at 55 °C overnight. The mixture was co-evaporated with toluene to dryness. The brown solid obtained was dissolved in 6 ml of ethyl acetate and 40 ml of ether was added to precipitate the product. After centrifugation, the liquid was poured out and the solid was washed with 30 ml of ether and dried to give small brown crystals (0.77 g, 82%). ESI MS: 277.11 ($M + H^+$), 299.09 ($M + Na^+$). ^1H NMR (DMSO-d_6) δ 8.67 (d, 1H), 8.04 (dd, 1H), 7.55 (d, 1H), 7.37 (s), 3.36 (m, 3H), 2.69 (q, 2H), 2.55 (q, 2H), 0.99 (t, 3H).

(B) Ethyl-(2-{[1-(5-nitro-1H-indol-2-yl)-methanoyl]-amino}-ethyl)-carbamic acid dimethyl-ethyl ester (**78**)

Compound **77** (0.12 g, 0.434 mmol) was dissolved in 3 ml of DMF and 0.48 ml of 1.0 M di-tert-butyl dicarbonate in THF was added. The reaction mixture was stirred at room temperature for 20 min until the reaction completed by TLC. The solvent was evaporated to dryness and a brown solid formed was recrystallized from MeOH-H_2O to give brown crystals (0.139 g, 85%). ESI MS: 377.15 ($M + H^+$), 399.13 ($M + Na^+$).

(C) 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-ethylamino-ethylcarbamoyl)-1H-indol-5-yl]-amide} (**76**)

Compound **78** (127 mg, 0.336 mmol) was reduced to **79** by hydrogenation according to general procedure A in example 10. A mixture of above amine DS13a and 1H-indole-2,5-dicarboxylic acid bis-(pentafluorophenyl-amide) **74** (45 mg, 0.084 mmol) in 2 ml of anhydrous DMF under argon was stirred at 55 °C overnight. The solvent was evaporated to dryness. The residue was dissolved in 5 ml of TFA/anisole (8:2) and the mixture was kept at room temperature for 1 h. The product was precipitated by ether and purified by HPLC described in general procedure B in example 10 to give **76** (22 mg, 40%). ESI MS: 662.27 ($M + H^+$), 331.64 ($M/2 + H^+$).

EXAMPLE 30

Synthesis of 1H-indole-2,5-dicarboxylic acid bis-{[2-(2-propylamino-ethylcarbamoyl)-1H-indol-5-yl]-amide} **80**

(A) 5-Nitro-1H-indole-2-carboxylic acid (2-propylamino-ethyl)-amide (**81**)

Similar procedure as described for preparation of **81** from 5-nitro-2-indolecarboxylic acid ethyl ester (0.8 g, 3.41 mmol) and N-propylethylenediamine (2 ml) gave a brown solid (0.84 g, 85%). ESI MS: 291.13 ($M + H^+$). ^{1}H NMR (DMSO-d_6) δ 8.68 (d, 1H), 8.04 (d, 1H), 7.55 (d, 1H), 7.37 (s, 1H), 3.35 (m, 3H), 2.68 (q, 2H), 2.49 9 (q, 2H), 1.40 (tt, 2H), 0.84 (t, 3H).

(B) (2-{[1-(5-Nitro-1H-indol-2-yl)-methanoyl]-amino}-ethyl)-propyl-carbamic acid dimethyl-ethyl ester (**82**)

Similar procedure as described for preparation of **78** from compound **81** (0.12 g, 0.413 mmol) gave brown powder (0.142 g, 88%). ESI MS: 391.17 ($M + H^+$), 413.15 ($M + Na^+$).

(C) 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-propylamino-ethylcarbamoyl)-1H-indol-5-yl]-amide} (**80**)

Compound **82** (131.2 mg, 0.336 mmol) was reduced to **83** by hydrogenation according to general procedure A in example 10. Similar procedure as described for the preparation of **76** from condensation of compound **83** with 1H-indole-2,5-dicarboxylic acid bis-

(pentafluorophenyl-amide) **74** (45 mg, 0.084 mmol) followed deprotection of Boc group and purification by HPLC gave GL496564 (39.4 mg, 63%). ESI MS: 690.31 ($M + H^+$), 345.66 ($M/2 + H^+$).

EXAMPLE 31

*Synthesis of 1-Octyl-1H-indole-2,5-dicarboxylic acid-***84**

Sodium hydride (60% suspension, 125 mg, 5 mmol) was added to a stirred solution of 1H-indole-2,5-dicarboxylic acid (525 mg, 2 mmol) in dry DMF (10 mL) and maintained at ambient temperature for 1 hour. The reaction was cooled to 0° C and then octyl bromide (1.5 mL, 13 mmol) was added. After 3 days the reaction was quenched by addition of 5% aqueous NH4Cl. The mixture was concentrated to dryness and then purified on a silica gel column using toluene. The product was then dissolved in 30 mL ethanol and 10 mL of 2 M NaOH was added. The solution was heated at 55° C for 2 days. The ethanol was removed *in vacuo* and the resulting aqueous solution was acidified with 0.01 M HCl to pH 3. The resulting precipitate was filtered and rinsed twice with water. The isolated product was dried by evaporation from absolute ethanol (3x) to give 460 mg (73%) of C1.
^{1}H NMR (DMSO): 8.44 (d, 1H, H-4 indole), 8.01 (dd, 1H, H-6 indole), 7.35 (m, 2 H, H-3&7 indole), 4.43-4.35 (m, 4H, Octyl), 1.4-1.2 (m, 10H, octyl), 0.865 (m, 3H, octyl)
MS: 316 [M-H]

EXAMPLE 32

*Synthesis of 1-Octyl-1H-indole-2,5-dicarboxylic acid dipentafluorophenyl ester-**85***

84 (460 mg, 1.45 mmol) and pentafluorophenol (560 mg, 3.045 mmol) was dissolved in dry DMF (7.25 mL) and then 628 mg (3.05 mmol) of dicyclohexylcarbodiimide dissolved in dry DMF (7.25 mL) was added. The reaction was maintained at ambient temperature for 3 days. The reaction was filtered through paper to remove precipitated urea and concentrated. The residue was taken up in EtOAc (50 mL) and filtered again. The solution was concentrated and then dissolved in 10 mL of dry dioxane and freeze-dried to afford C2 (823 mg, 87%).

EXAMPLE 33

Synthesis of 1-Octyl-1H-indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}-**86**

90 mg (0.325 mmol) of freshly reduced (as described above) "amino-pyrrole(N1-methyl) amidine" was dissolved in dry DMF (1.25 mL) and added to 65 mg (0.1 mmol) of **84**. The reaction was maintained at 40° C for 3 days. The product was precipitated with 40 mL cold diethyl ether, decanted and rinsed once more with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **86** as the bis-trifluoroacetate salt. This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **86** as the bis-HCl salt (13.1 mg).

MS: 350.7 [M+2H]/2

EXAMPLE 34

Synthesis of 1-Propyl-1H-indole-2,5-dicarboxylic acid -**87**

Sodium hydride (60% suspension, 125 mg, 5 mmol) was added to a stirred solution of 1H-indole-2,5-dicarboxylic acid (525 mg, 2 mmol) in dry DMF (10 mL) and maintained at ambient temperature for 1 hour. The reaction was cooled to 0° C and then propyl bromide (0.275 mL, 3 mmol) was added. After 3 days the reaction was quenched by addition of 5% aqueous NH_4Cl. The mixture was concentrated to dryness and then purified on a silica gel column using 5% EtOAc/toluene. The product was then dissolved in 30 mL ethanol and 10 mL of 2 M NaOH was added. The solution was heated at 55° C for 2 days. The ethanol was removed *in vacuo* and the resulting aqueous solution was acidified with 0.01 M HCl to pH 3. The resulting precipitate was filtered and rinsed twice with water. The isolated product was dried by evaporation from absolute ethanol (3x) to give 340 mg (67%) of **87**.

^{1}H NMR ($CDCl_3$): 8.38 (s, 1H, H-4 indole), 7.87 (d, 1H, H-6 indole), 7.71 (d, 1 H, H-7 indole), 7.44 (d, 1 H, H-3 indole), 4.53 (m, 2H, Propyl), 1.7 (m, 2H, propyl), 0.81 (m, 3H, propyl)

MS: 246 [M-H]

EXAMPLE 35

Synthesis of 1-Propyl-1H-indole-2,5-dicarboxylic acid dipentafluorophenyl ester-**88**

84 (340 mg, 1.33 mmol) and pentafluorophenol (514 mg, 2.8 mmol) was dissolved in dry DMF (6.65 mL) and then 575 mg (2.8 mmol) of dicyclohexylcarbodiimide dissolved in dry DMF (6.65 mL) was added. The reaction was maintained at ambient temperature for 3 days. The reaction was filtered through paper to remove precipitated urea and concentrated. The residue was taken up in EtOAc (50 mL) and filtered again. The solution was concentrated and then dissolved in 10 mL of dry dioxane and freeze-dried to afford **88** (626 mg, 81%).

EXAMPLE 36

Synthesis of 1-Propyl-1H-indole-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-methyl-1H-pyrrol-3-yl]-amide}--**89**

90 mg (0.325 mmol) of freshly reduced (as described above) amino-pyrrole(N1-methyl) amidine was dissolved in dry DMF (1.25 mL) and added to 58 mg (0.1 mmol) of **88**. The reaction was maintained at 40° C for 3 days. The product was precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 μM C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **89** as the bis-trifluoroacetate salt. This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **89** as the bis-HCl salt (19.3 mg).

MS: 315.7 [M+2H]/2

EXAMPLE 37

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-methylamino-ethylcarbamoyl)-1H-indol-5-yl]-amide}-**90**

To 30 mg (0.05 mmol) of "EtO-Ind-Ind-Ind-OEt" was added 1.5 mL of N-methylethylenediamine. The mixture was reacted at 50° C for 72 hours and then concentrated *in vacuo*. The residue was taken up into 2 mL DMF and precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 μM C_{18} 2.2x25 cm column, 0% to 60%

acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **90** as the bis-trifluoroacetate salt. This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **90** as the bis-HCl salt (11.5 mg).

MS: 317.7 [M+2H]/2

EXAMPLE 38

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-[(2-{2-[bis-(2-amino-ethyl)-amino]-ethylcarbamoyl}-1H-indol-5-yl)-amide]-**91**

To 50 mg (0.087 mmol) of "EtO-Ind-Ind-Ind-OEt" was added 3 mL of Tris-(2-aminoethyl)amine. The mixture was reacted at 55° C for 24 hours and then precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **91** as the hexa-trifluoroacetate salt. (22.9 mg).

MS: 389.7 [M+2H]/2

EXAMPLE 39

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-({2-[3-(3-amino-propylamino)-propylcarbamoyl]-1H-indol-5-yl}-amide)-**92**

To 50 mg (0.087 mmol) of "EtO-Ind-Ind-Ind-OEt" was added 3 mL of 3-aminopropyl-propane-diamine and 1 mL DMF. The mixture was reacted at 55° C for 48 hours and then precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **92** as the tetrakis-trifluoroacetate salt. (20.4 mg).

MS: 374.7 [M+2H]/2

EXAMPLE 40

Synthesis of 5-Nitro-1-propyl-1H-indole-2-carboxylic acid ethyl ester-**93**

To 3.69 g (15.75 mmol) of commercial ethyl 5-Nitro-2-carboxy-indole dissolved in 35 mL of DMSO was added 2.01 g (31.5 mmol) of KOH. The reaction was stirred vigourously for 30 mins., at which time 2.86 mL (31.5 mmol) of propyl bromide was added. After 4 hours an additional 5 mL DMSO was added and the reaction was reacted overnight. 1 mL 5% aqueous NH4Cl was added and poured into toluene (150 mL) and washed with saturated $NaHCO_3$ (100 mL). The aqueous layer was extracted twice with toluene (75 mL each) and the combined organic layers washed with brine (100 mL), dried over Na_2SO_4 and concentrated. The product was dissolved in 100 mL dioxane and freeze-dried to give 4.15 g (15.1 mmol, 95%) of **93**.

^1H NMR ($CDCl_3$): 8.64 (s, 1H, H-4 indole), 8.2 (m, 1H, H-6 indole), 7.47-7.42 (m, 2 H, H-7 & H-3 indole), 4.57 (m, 2H, Propyl), 4.45-4.37 (m, 4H, ethyl ester), 1.89-1.8 (m, 2H, propyl), 1.47-1.41 (m, 3H, propyl), 0.98-0.98-0.92 (m, 6H, ethyl ester)

MS: 299 [M+Na]

EXAMPLE 41

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-propyl-1H-indol-5-yl]-amide}-**94**

To a solution of **93** (104 mg, 0.375 mmol) in 50 mL anhydrous EtOAc and 25 mL anhydrous methanol was added 10% Pd/C (Degussa type, Aldrich) (0.05 g). The flask was evacuated and flushed with hydrogen three times and finally filled with hydrogen at 40 psi. The suspension was shaken vigourously for 45 mins. at ambient temperature. The suspension was filtered through a Buchner funnel and rinsed several times with methanol. The filtrate and washings were concentrated to dryness. The resulting amino-indole was then dissolved in dry DMF (1 mL) and added to 75 mg (0.15 mmol) "Pfp-Indole-Pfp" in a vial and placed at 55° C for 24 hours. The crude tris-indole was isolated by addition of 40 mL of 0.001 M HCl to the reaction mixture. The precipitate was isolated by centrifugation and the acidic supernatant decanted. The crude was rinsed and centrifuged once more with 0.001 M HCl and three times with water. The product was dried by evaporation twice from ethanol. Finally, the crude residue was placed in a vial and 2 mL redistilled ethylenediamine was added. The

reaction was heated at 55° C for 72 hrs. and then concentrated *in vacuo*. The residue was taken up into 2 mL DMF and precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **94** as the bis-trifluoroacetate salt This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **94** as the bis-HCl salt (30.0 mg).
MS: 345.7 [M+2H]/2

EXAMPLE 42

Synthesis of 1-Propyl-1H-indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-propyl-1H-indol-5-yl]-amide}-**95**

To a solution of **93** (104 mg, 0.375 mmol) in 50 mL EtOAc and 25 mL methanol was added 10% Pd/C (Degussa type, Aldrich) (0.05 g). The flask was evacuated and flushed with hydrogen three times and finally filled with hydrogen at 40 psi. The suspension was shaken vigourously for 45 mins. at ambient temperature. The suspension was filtered through a Buchner funnel and rinsed several times with methanol. The filtrate and washings were concentrated to dryness. The resulting amino-indole was then dissolved in dry DMF (1 mL) and added to 87 mg (0.15 mmol) **88** in a vial and placed at 55° C for 24 hours. The crude tris-indole was isolated by addition of 40 mL of 0.001 M HCl to the reaction mixture. The precipitate was isolated by centrifugation and the acidic supernatant decanted. The crude was rinsed and centrifuged once more with 0.001 M HCl and three times with water. The product was dried by evaporation twice from ethanol. Finally, the crude residue was placed in a vial and 2 mL redistilled ethylenediamine was added. The reaction was heated at 55° C for 72 hrs. and then concentrated *in vacuo*. The residue was taken up into 2 mL DMF and precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **95** as the bis-trifluoroacetate salt. This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **95** as the bis-HCl salt (48.5 mg).

MS: 366.7 [M+2H]/2

EXAMPLE 43

Synthesis of 1-Propyl-1H-indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}-**96**

To a solution of commercial ethyl 5-Nitro-2-carboxy-indole (87 mg, 0.37 mmol) in 75 mL EtOAc and 25 mL methanol was added 10% Pd/C (Degussa type, Aldrich) (0.05 g). The flask was evacuated and flushed with hydrogen three times and finally filled with hydrogen at 40 psi. The suspension was shaken vigourously for 45 mins. at ambient temperature. The suspension was filtered through a Buchner funnel and rinsed several times with methanol. The filtrate and washings were concentrated to dryness. The resulting amino-indole was then dissolved in dry DMF (1 mL) and added to 87 mg (0.15 mmol) C5 in a vial and placed at 55° C for 24 hours. The crude tris-indole was isolated by addition of 40 mL of 0.001 M HCl to the reaction mixture. The precipitate was isolated by centrifugation and the acidic supernatant decanted. The crude was rinsed and centrifuged once more with 0.001 M HCl and three times with water. The product was dried by evaporation twice from ethanol. Finally, the crude residue was placed in a vial and 3 mL redistilled ethylenediamine was added. The reaction was heated at 55° C for 72 hrs. and then concentrated *in vacuo*. The residue was taken up into 1 mL DMF and precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **96** as the bis-trifluoroacetate salt. This was dissolved in 2 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **96** as the bis-HCl salt (7.7 mg).

MS: 324.7 [M+2H]/2

EXAMPLE 44

Synthesis of 5-Nitro-1H-indole-2-carboxylic acid-**97**

To a solution of commercial ethyl 5-Nitro-2-carboxy-indole (10.21 g, 43.6 mmol) in ethanol (220 mL) was added 110 mL of 2 M NaOH. The reaction was stirred at 60° C for 18 hours. The reaction was then cooled and the ethanol removed *in vacuo* and then an additional 200 mL water was added. To the vigourously stirring aqueous solution was added 5 M HCl

followed by 1 M HCl until pH 4 was attained and the acid product was precipitated. The product was collected by filtration on a Buchner funnel and washed once with dilute HCl (1:40 v/v) and twice with water. The filtrate was dried over P_2O_5 in a dessicator *in vacuo* to afford 8.83 g (42.8, 98%) of acid **97**.

^{1}H NMR (DMSO): 12.4 (br s, 1H, 1H indole), 8.68 (dd, 1H, H-4 indole), 8.08 (m, 1H, H-6 indole), 7.54 (m, 1H, H-7 indole), 7.34 (dd, 1H, H-3 indole).

EXAMPLE 45

*Synthesis of 5-Nitro-1H-indole-2-carboxylic acid (2-cyano-ethyl)-amide-***98**

4.5 g (21.8 mmol) of acid **97** was placed in a flask and 100 mL of thionyl chloride was added. The reaction was refluxed at 85° C for 2.5 hrs under a dry atmosphere. The reaction was cooled to ambient temperature and the mixture concentrated *in vacuo*. The residue was taken up into 75 mL dioxane and the suspension concentrated *in vacuo*. Finally, the residue was taken up into 100 mL toluene and the suspension concentrated *in vacuo*. The residue was suspended in dry dioxane (220 mL) and amino-propionitrile (3.96 mL, 54.5 mmol) was added dropwise. The reaction was stirred at ambient temperature for 18 hrs. and then concentrated *in vacuo*. The residue was taken into 50 mL DMF and with vigourous stirring 0.001 M HCl was added until pH 3 was attained and then an additional 200 mL 0.001 M HCl was added. The product was collected by filtration on a Buchner funnel and washed twice with water. The filtrate was dried over P_2O_5 in a dessicator *in vacuo* to afford 5.13 g (19.9, 91%) of **98**.

^{1}H NMR (DMSO): 12.34 (br s, 1H, 1H indole), 9.1 (dd, 1H, H-4 indole), 8.7 (s, 1H, amide NH), 8.04 (m, 1H, H-6 indole), 7.55 (m, 1H, H-7 indole), 7.38 (s, 1H, H-3 indole), 3.54-3.49 (m, 2H), 2.78 (dd, 2H).

EXAMPLE 46

*Synthesis of 5-Nitro-1H-indole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amide-***99**

Nitrile **98** (2.5 g, 9.68 mmol) was suspended in anhydrous ethanol (75 mL) and cooled to 0° C. The cooled ethanolic suspension was then saturated with dried HCl gas for 5 hours. The gas stream was removed, the flask sealed and kept overnight at 4° C. In the morning, the suspension was concentrated *in vacuo* and then coevaporated with anhydrous

ethanol (100 mL) to afford 3.26 g of imidate ester intermediate. 2.26 g of the crude was suspended in anhydrous ethanol (100 mL), cooled to 0° C and saturated with anhydrous NH_3 gas. After 4 hrs. the gas source was removed, the flask sealed and placed at 4° C overnight. In the morning, the reaction was concentrated *in vacuo* and coevaporated once with anhydrous ethanol (100 mL). The residue was suspended in anhydrous ethanol (200 mL), filtered, rinsed with anhydrous ethanol and dried *in vacuo* to afford 1.89 g (5.86 mmol) of **99**.

^{1}H NMR (DMSO): 9.1-9.05 (br m, 3H, H4 indole & amidine), 8.75-8.65 (br s, 2 H, amidine), 8.06 (dd, 1H, H-6 indole), 7.57 (d, 1H, H-7 indole), 7.45 (s, 1H, H-3 indole)3.65-3.61 (m, 2H), 2.71-2.66 (m, 2H).

MS: 276 [M+H]

EXAMPLE 47

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-carbamimidoyl-ethylcarbamoyl)-1H-indol-5-yl]-amide}-**100**

To a solution of "nitro-indole-amidine" **99** (117mg, 0.375 mmol) in methanol (30 mL) and EtOAc (10 mL) was added 10% Pd/C (Degussa type, Aldrich) (0.05 g). The flask was evacuated and flushed with hydrogen three times and finally filled with hydrogen at 50 psi. The suspension was shaken vigourously for 45 mins. at ambient temperature. The suspension was filtered through a Buchner funnel and rinsed several times with methanol. The filtrate and washings were concentrated to dryness. The resulting amino-indole was then dissolved in dry DMF (1.9 mL) and added to 75 mg (0.15 mmol) "Pfp-Indole-Pfp" in a vial and placed at 45° C for 48 hours. The product was precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. The crude product was taken up into 0.1% aqueous TFA and purified by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **100** as the bis-trifluoroacetate salt. This was dissolved in 6 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **100** as the bis-HCl salt (62.0 mg).

MS: 330.6 [M+2H]/2

EXAMPLE 48

Synthesis of 9H-Carbazole-3,6-dicarboxylic acid dipentafluorophenyl ester-**101**

Commercial carbazole (5.02 g, 30 mmol) was suspended in chlorobenzene (48 mL) and trichloroacetonitrile (7.2 mL, 72 mmol) added. $AlCl_3$ was then added to the stirring reaction mixture. The reaction mixture was fit with reflux condensor under a dry atmosphere and slowly heated to 100° C. After 2 hrs. 20 mL concentrated HCl was added and the temperature increased to 120° C for 2 hours. The mixture was concentrated *in vacuo* and then suspended in 2 M KOH (200 mL), refluxed for 1 hour and finally, filtered through a Buchner funnel. The filtrate was adjusted to pH 3 with 5 M HCl, cooled to ambient temperature and filtered. The filtrate was dried by coevaporation from methanol three times to afford 1.86 g of crude diacid.

The crude diacid was dissolved in and concentrated from anhydrous pyridine three times and dissolved in dry DMF (14.5 mL). Diisopropylethylamine (5.05 mL) was added followed by 2.62 mL (15.25 mmol) of pentafluorophenyl-trifluoroacetate. The reaction was stirred at ambient temperature overnight and then concentrated *in vacuo*. The residue was then purified on a silica gel column using 50% EtOAc/toluene. The product was dissolved in anhydrous benzene (30 mL) and freeze dried to afford 290 mg (0.494 mmol, 7%) of **101**. ^1H NMR ($CDCl_3$): 9.04 (s, 2H, H-4&5), 8.3 (dd, 2H, H-1&8), 7.64 (d, 2H, H-2&7).

EXAMPLE 49

9H-Carbazole-3,6-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}-**102**

To a solution of "5-nitro-indole-EDA-Boc" (74 mg, 0.21 mmol) in methanol (25 mL) and EtOAc (25 mL) was added 10% Pd/C (Degussa type, Aldrich) (0.01 g). The flask was evacuated and flushed with hydrogen three times and finally filled with hydrogen at 40 psi. The suspension was shaken vigourously for 45 mins. at ambient temperature. The suspension was filtered through a Buchner funnel and rinsed several times with methanol. The filtrate and washings were concentrated to dryness. The resulting amino-indole was then dissolved in dry DMF (1.0 mL) and added to 57 mg (0.1 mmol) "Pfp-Carbazole-Pfp"-**101** in a vial and placed at 50° C for 20 hours. The Boc-protected product was precipitated with 40 mL cold diethyl ether, decanted and rinsed once with ether. To the residue was added anisole (0.8 mL) and then trifluoroacetic acid (3.2 mL). The solution was maintained at ambient temperature for 30 minutes and then product was precipitated with 40 mL cold diethyl ether, decanted and rinsed twice with ether. The crude product was taken up into 0.1% aqueous TFA and purified

by HPLC (Vydac 12 M C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, 20 mL/min.) to afford **102** as the bis-trifluoroacetate salt. This was dissolved in 6 mL dry MeOH, cooled to –20° C and then 1 mL 4 M HCl/dioxane was added. The solution was precipitated with 40 mL cold ether to afford **102** as the bis-HCl salt (38.4 mg).
MS: 328.7 [M+2H]/2

EXAMPLE 50

Synthesis of 1H-Indole-2,5-dicarboxylic acid 2-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide} 5-{[2-(2-guanidino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **103**, *(Scheme 6)*

Loading of the linker. 2.5 g (2.55 mmol) MBHA resin (S=1.02) was swelled in DMF for 5 minutes. 1.06g (7.65 mmol) 4-hydroxybenzoic acid and 1.03 g (7.65 mmol) HOBt was dissolved in DMF to which 1.18 mL (7.65 mmol) DIC was added. The clear mixture was poured to the resin and was agitated gently for 2 hrs. The resin was drained, washed with DMF (5x). A mixture of 10 mL DMF and 5 mL ethanolamine was added and was agitated overnight at room temperature (18 hrs). Next morning the resin was drained, washed with DMF (3x), DCM (3x), 50% TFA/DCM (2x), DCM (2x), DMF (2x), DCM (2x), methanol (2x), ether (2x) and it was dried to get 2.8 g phenol resin, B1. The degree of substitution was S=0.89 mmol/g resin (calculated from the weight increase).

Loading of the first acid. 1.2 g (5 mmol) N-*tert*-butyloxycarbonyl-5-aminoindole-2-carboxylic acid (Boc-5Ain-OH) was suspended in 20 mL DCM. 0.78 mL (5 mmol) DIC was added followed by 100 mg (0.8 mmol) DMAP. The suspension became clear within 5 minutes. The clear solution was added to the dry, 2.8 g (2.5 mmol) phenol resin, B1 and the mixture was agitated overnight (18 hrs) at room temperature. Next day the resin was drained, washed with DMF (3x). The unreacted phenolic OH groups were blocked by acetylation with 20% acetic anhydryde in DCM plus 0.5 mL DIEA. The resin was then washed with DMF (3x), DCM (3x), methanol (2x), ether (2x) and was dried resulting in 3.2 g B2. The degree of substitution was about 0.55 mmol/g resin – based on the weight increment.

Synthesis. 160 mg (0.1 mmol) Boc-5Ain-Hba-Resin (B2) was swelled in DCM for ten minutes then was treated with 25% TFA 2% anisol in DCM for 20 minutes. It was washed 3x with DCM and 3x with DMF. The unprotected B3 was coupled in DMF with 151 mg (0.3 mmol) B4 dipeptide (synthesized separately in solution) using 108 mg (0.285 mmol) HBTU and 104 μL (0.6 mmol) DIEA for three hrs resulting in the resin bound tripeptide, B5. The

resin was washed with DMF (3x), DCM (3x) and was treated with the TFA/anisol/DCM reagent again for 20 minutes. The TFA was washed out with DCM (3x) and DMF (3x). The free amino containing molecule was treated with 10 fold excess of 1*H*-Pyrazole-1-carboxamidine hydrochloride (146.6 mg, 1.0 mmol) and DIEA (344 μL, 2.0 mmol) overnight at room temperature to give B6. Finally, the product (**103**) was cleaved from the resin by treating with EDA at room temperature for 1 hour. The resin was filtered off, the supernatant was evaporated in vacuum and the remaining oil was precipitated from methanol with ether. The precipitate was spun down and was dried. The crude product was purified with HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The overall yield was 16.4 mg (24%) **103**. ES MS: 648.26 (calcd. for $M+H^+$: 648.28).

EXAMPLE 51

Synthesis of 1H-Indole-2,5-dicarboxylic acid 5-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide} 2-{[2-(2-guanidino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **104**

The same amount of tBoc protected peptide-resin (0.1 mmole, Scheme [**FILL IN**], B5), instead of removing the protecting group was first cleaved from the resin with EDA as described in Example 50. The resulted amine was treated with 146.6 mg (1.0 mmole) of 1*H*-Pyrazole-1-carboxamidine hydrochloride in DMF (2mL) solution overnight. The reaction mixture was evaporated to dryness and the remaining oil was dissolved in 5 mL TFA containing 20% anisol. The deprotection was complete in 30 minutes, when the product was precipitated by addition of 45 mL cold diethylether. The precipitate was filtered off, was washed with ether and was dried. The crude product was purified with HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The overall yield was 15.2 mg (22%) **104**. ES MS: 648.26 (calcd. for $M+H^+$: 648.28).

EXAMPLE 52

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-guanidino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **105**

8.2 mg (0.01 mmole) **103** was treated with 14.6 mg (0.1 mmole) of 1*H*-Pyrazole-1-carboxamidine hydrochloride in DMF (2mL) solution as described in Example 51. After evaporation, the oily residue was purified with HPLC in the same way. Yield: 15.6 mg (23%) B9. ES MS: 690.27 (calcd. for $M+H^+$: 690.30).

EXAMPLE 53

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **106**

(A) 6-Amino-1H-indole-2-carboxylic acid methyl ester, **107** ($R=CH_3$).

To a solution of 6-Nitro-1H-indole-2-carboxylic acid methyl ester (**108**) 200 mg (0.91 mmole) in a mixture of methanol/ethylacetate 1:1 10% Pd/C (40mg) was added. The flask was rinsed 3 times with hydrogen and filled with hydrogen at 30 to 35 psi. The suspension was stirred vigorously at room temperature for 30 minutes. The catalyst was filtered off, the filtrate was evaporated *in vacuo* to dryness. The resulted 6-amino-1H-indole-2-carboxylic acid methyl ester gave a single spot on TLC (Silica, toluene-ethylacetate 7:3, R_f: 0.31) and was used for the next step without purification

(B) 1H-Indole-2,5-dicarboxylic acid bis-{[2-methoxycarbonyl-1H-indol-6-yl]-amide}, **109** ($R=CH_3$)

The freshly prepared (as described above) 6-amino-1H-indole-2-carboxylic acid methyl ester (0.91 mmole) was dissolved in 3 mL of dry DMF. 235 mg (0.44 mmole) 1H-Indole-2,5-dicarboxylic acid dipentafluorophenyl ester, **110** (Example 1, Step B) and 156 µL (0.91 mmole) DIEA were added and the mixture was heated under argon at 55 C° for three days then was evaporated to dryness. The oily residue was triturated with ether to give 200 mg (83%) solid product which was pure enough to continue the synthesis without further purification.

(C) 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **106**

50 mg (0.091 mmole) **109** ($R=CH_3$) was dissolved in 2 mL neat 1,2-ethylenediamine and was kept at 55 C° overnight (18 hrs) and was evaporated to dryness. The residue was

dissolved in 2 mL methanol and was precipitated by addition of 45 mL of ether. The precipitate was spun down, the pallet was washed twice with ether and was dried. The crude **106** was purified with HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The purified compound was transferred to HCl salt by dissolving in 2 mL methanol, treating with 1 mL 4N HCl in dioxane and precipitating with ether. The overall yield was 9.1 mg (15%) **106**. ES MS: 606.30 (calcd. for $M+H^+$: 606.26).

EXAMPLE 54

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(3-amino-propylcarbamoyl)-1H-indol-6-yl]-amide}, **111**

Compound **111** was synthesized as described for Compound **106** in Example 53, using propane-1,3-diamine in Step C. Yield 8.6 mg (18%); MS: 634.38 (calcd. for $M+H^+$: 634.29).

EXAMPLE 55

Synthesis of N,N'-Bis-[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-isophthalamide, **112**

Compound **112** was synthesized as generally described for Compound **106** in Example 53. Yield 10.9 mg (18%); MS: 567.26 (calcd. for $M+H^+$: 567.25).

EXAMPLE 56

Synthesis of Pyridine-2,6-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **113**

Compound **113** was synthesized as generally described for Compound **106** in Example 53. Yield 23.2 mg (41%); MS: 568.24 (calcd. for $M+H^+$: 568.24).

EXAMPLE 57

Synthesis Pyridine-2,4-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **114**

Compound **114** was synthesized as generally described for Compound **106** in Example 53. Yield 17.1 mg (30%); MS: 568.24 (calcd. for $M+H^+$: 568.24).

EXAMPLE 58

Synthesis Pyridine-3,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **115**

Compound **115** was synthesized as generally described for Compound **106** in 53. Yield 30 mg (53%); MS: 568.25 (calcd. for $M+H^+$: 568.24).

EXAMPLE 59

Synthesis N,N'-Bis-[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-isophthalamide, **116**

Compound **116** was synthesized as generally described for Compound **106** in Example 53. Yield 34.9 mg (61%); MS: 567.26 (calcd. for $M+H^+$: 567.25).

EXAMPLE 60

Synthesis Pyridine-2,6-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **117**

Compound **117** was synthesized as generally described for Compound **106** in Example 53. Yield 35.5 mg (61%); MS: 568.25 (calcd. for $M+H^+$: 568.24).

EXAMPLE 61

Synthesis Pyridine-2,4-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **118**

Compound **118** was synthesized as generally described for Compound **106** in Example 53. Yield 35.3 mg (61%); MS: 568.26 (calcd. for $M+H^+$: 568.24).

EXAMPLE 62

Synthesis 1H-Pyrazole-3,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **119**

Compound **119** was synthesized as generally described for Compound **106** in Example 53. Yield 9.6 mg (17%); MS: 557.23 (calcd. for $M+H^+$: 557.24).

EXAMPLE 63

Synthesis Thiophene-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **120**

Compound **120** was synthesized as generally described for Compound **106** in Example 53. Yield 8.7 mg (15%); MS: 573.19 (calcd. for $M+H^+$: 573.21).

EXAMPLE 64

Synthesis 1H-Pyrazole-3,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **121**

Compound **121** was synthesized as generally described for Compound **106** in Example 53. Yield 3.7 mg (7%); MS: 557.23 (calcd. for $M+H^+$: 557.24).

EXAMPLE 65

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl]-1-methyl-1H benzimidazole-5-yl]-amide}, **122**

Compound **122** was synthesized as generally described for Compound **106** in Example 53. Yield 6.8mg (10%); MS: 636.37 (calcd. for $M+H^+$: 636.40).

EXAMPLE 66

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-({2-[2-(2-hydroxy-ethylamino)-ethylcarbamoyl]-1H-indol-6-yl}-amide), **123**

Compound **123** was synthesized as generally described for Compound **106** in 53. Yield 12.5mg (17%); MS: 694.35 (calcd. for $M+H^+$: 694.31).

EXAMPLE 67

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-dimethylamino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **124**

(A) Synthesis of 6-Nitro-1H-indole-2-carboxylic acid (2-dimethylamino-ethyl)-amide, **125**

500 mg (2.27 mmole) 6-Nitro-1H-indole-2-carboxylic acid methyl ester was dissolved in 4 mL neat N^1,N^1-dimethyl-ethane-1,2-diamine, was kept at 55 C° overnight and was evaporated. The oily residue was triturated with n-hexane to give 528 mg (84%) yellow solid which was no further purified. MS: 277.13 (calcd for $M+H^+$: 277.13)

(B) Synthesis of 6-Amino-1H-indole-2-carboxylic acid (2-dimethylamino-ethyl)-amide, **126**

To a solution of 82.9 mg (0.3 mmole) **125** in a mixture of ethanol/ethylacetate 1:1 10% Pd/C (40mg) was added. The flask was flushed 3 times with hydrogen and filled with hydrogen at 30 to 35 psi. The suspension was stirred vigorously at room temperature for 30 minutes. The catalyst was filtered off, the filtrate was evaporated *in vacuo* to dryness. The solid **126** was used for the next step without purification

(C) 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-dimethylamino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **124**

The freshly prepared (as described above) **126** was dissolved in 3 mL dry DMF. 54 mg (0.1 mmole) 1-H-indole-2,5-dicarboxylic acid dipentafluorophenyl ester (Example 1, Step B) and 103 µL (0.6 mmole) DIEA were added and the mixture was heated under argon at 55 C° overnight (18 hrs) then was evaporated to dryness. The crude **124** was purified with HPLC (Vydac 12 µm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The purified compound was converted to HCl salt by dissolving in 2 mL methanol, treating with 1 mL 4N HCl in dioxane and precipitating with ether to yield 7.0 mg (9.5 %) **124**. ES MS: 662.29 (calcd. for $M+H^+$: 662.32).

EXAMPLE 68

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(3-dimethylamino-propylcarbamoyl)-1H-indol-5-yl]-amide}, **127**

Compound **127** was synthesized as generally described for Compound **124** in Example 67. Yield 16.7 mg (24%); MS: 690.34 (calcd. for $M+H^+$: 690.35).

EXAMPLE 69

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-dimethylamino-ethylcarbamoyl)-2,3-dihydro-1H-indol-6-yl]-amide}, **128**

Compound **128** was synthesized as generally described for Compound **124** in Example 67. Yield 4.8 mg (7%); MS: 666.33(calcd. for $M+H^+$: 666.35).

EXAMPLE 70

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-dimethylamino-propylcarbamoyl)-2,3-dihydro-1H-indol-6-yl]-amide}, **129**

Compound **129** was synthesized as described for Compound **124** in Example 67. Yield 23.7 mg (34%); MS: 694.36 (calcd. for $M+H^+$: 694.39).

EXAMPLE 71

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-({2-[2-(2-hydroxy-ethylamino)-ethylcarbamoyl]-1H-indol-5-yl}-amide), **130**

(A) 5-Nitro-1H-indole-2-carboxylic acid [2-(2-hydroxy-ethylamino)-ethyl]-amide, **131**

To a solution of 2.34 g (10 mmole) 5-Nitro-1H-indole-2-carboxylic acid ethyl ester, (**132**) in 25 mL DMF 5.2 g (50 mmole) 2-(2-amino-ethylamino)-ethanol (**133**) was added and the mixture was kept at 55 °C for 36 hrs under argon atmosphere. It was then evaporated to dryness and the oily residue was dissolved at room temperature in ethanol resulting in an immediate crystal formation. The crystals were filtered off, washed with ethanol (2x) and dried to give 2.08g product (71%). MS: 293.13 (calcd for $M+H^+$: 293.31). ^{1}H-NMR (DMSO-d_6): 8.70-8.67 (m, 2H, amide & indole H-4); 8.04 (dd, 1H, indole H-6); 7.55 (d, 1H, indole H-7); 7.37 (s, 1H, indole H-3); 3.43 (t, 2H, -NH-CH_2-CH_2-NH-CH_2-$C\underline{H}_2$-OH); 3.39-3.33 (m, 2H, -NH-$C\underline{H}_2$-CH_2-NH-CH_2-CH_2-OH); 2.71-2.66 (m, 2H, -NH-CH_2-$C\underline{H}_2$-NH-CH_2-CH_2-OH); 2.59 (t, 2H, -NH-CH_2-CH_2-NH-$C\underline{H}_2$-CH_2-OH)

(B) 5-Nitro-1H-indole-2-carboxylic acid [(2-(2-hydroxy-ethyl-2-*tert*-butyloxycarbonyl-amino)-ethyl]-amide, **134**

2.08g (7.12 mmole) **131** was suspended in 10 mL DMF. 1.71 g (7.83 mmole) $tBoc_2O$ was added at room temperature. The mixture became clear in ten minutes and the reaction was complete in 1 hr. The DMF was evaporated in vacuo, the remaining solid was crystallized from iso-propanol to yield 1.82g (65%). ^{1}H-NMR (DMSO-d_6):12.33 (s, 1H, indole H-1); 8.79 (s, 1H, CO-NH); 8.69 (s, 1H, indole H-3); 8.03 (dd, 1H, indole H-6); 7.55 (d, 1H, indole H-7); 7.34 (d, 1H, indole H-4); 3.46-3.23 (m, 8H, methylenes); 1.32 (s, 9H, CH_3)

(C) 5-Amino-1H-indole-2-carboxylic acid [(2-(2-hydroxy-ethyl-2-*tert*-butyloxycarbonyl-amino)-ethyl]-amide, **135**

To a solution of 196 mg (0.5 mmole) **134** in a mixture of ethanoll/ethylacetate 1:1 10% Pd/C (50mg) was added. The flask was rinsed 3 times with hydrogen and filled with hydrogen at 30 to 35 psi. The suspension was stirred vigorously at room temperature for 30 minutes. The catalyst was filtered off, the filtrate was evaporated *in vacuo* to dryness to result in 180mg (100%) **135** that gave a single spot on TLC (Silica, toluene-ethylacetate 1:9, R_f: 0.16) and was used for the next step without purification.

(D) 1H-Indole-2,5-dicarboxylic acid bis-({2-[2-(2-hydroxy-ethylamino)-ethylcarbamoyl]-1H indol-5-yl}-amide), **130**

180 mg (0.5 mmole) **135** was dissolved in 3 mL DMF and was reacted with 50 mg (0.1mmole) 1-H-indole-2,5-dicarboxylic acid dipentafluorophenyl ester and 86 µL (0.5 mmole) DIEA overnight (18 hrs) at 55 °C. The mixture was evaporated to drynes in vacuo, the semisolid remaining was triturated with ether to give 190 mg solid **136**. The tBoc protecting groups were removed by dissolving it in 5 mL TFA containing 20% anisol and reacting for 30 minutes at room temperature. 40 mL ether was added and the mixture was spun down. The supernatant was discarded, the pallet was washed with ether 3 times and was dried. The crude **130** was purified with HPLC (Vydac 12 µm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The purified compound was converted to HCl salt by dissolving in 2 mL methanol, treating with 1 mL 4N HCl in dioxane

and precipitating with ether to yield 29.3 mg (42.3 %) **130**. ES MS: 694.29 (calcd. for $M+H^+$: 694.31).

EXAMPLE 72

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-{[2-(3-amino-2-hydroxy-propylcarbamoyl]-1H indole-5-yl]-amide}, **137**

Compound **137** was synthesized as generally described for Compound **130** in Example 71. Yield 58mg (86%); MS: 666.42 (calcd. for $M+H^+$: 666.28).

EXAMPLE 73

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-guanidino-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **138**

To a solution of 23.8 mg (35 μmole) **106** (synthesized as described in example 53) in 2 mL of DMF 51 mg (0.35 mmole) 1-H-pyrazole-1-carboxamidine hydrochloride and 73 μL (0.42 mmole) DIEA was added. The mixture was kept overnight (18 hrs) at room temperature then was evaporated to dryness. The oily residue was purified with HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The purified compound was converted to HCl salt by dissolving in 2 mL methanol, treating with 1 mL 4N HCl in dioxane and precipitating with ether to yield 3.3 mg (13 %) **138**. ES MS: 690.41 (calcd. for $M+H^+$: 690.30).

EXAMPLE 74

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-guanidino-propylcarbamoyl)-1H-indol-6-yl]-amide}, **139**

Compound **139** was synthesized as generally described for Compound **138** in Example 73. Yield 2.0mg (8%); MS: 718.43 (calcd. for $M+H^+$: 718.33).

EXAMPLE 75

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-guanidino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **140**

Compound **140** was synthesized as generally described for Compound **138** in Example 53. Yield 50 mg (24%); MS: 690.39 (calcd. for $M+H^+$: 690.30).

EXAMPLE 76

Synthesis 1H-Indole-2,5-dicarboxylic acid bis-{[2-(3-guanidino-2-hydroxy-propylcarbamoyl]-1H indole-5-yl]-amide}, **141**

Compound **141** was synthesized as described for Compound **138** in Example 53. Yield 9.1 mg (60%); MS: 750.37 (calcd. for $M+H^+$: 750.32).

EXAMPLE 77

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-ethoxymethyl-1H-indol-5-yl]}-amide}, **142**

A solution of 2.3 g (10 mmole) 5-nitro-1H-indole-2-carboxylic acid ethyl ester (**143**) in DMF was cooled to 0 °C and 598 mg (15 mmole) NaH (60% in mineral oil) was added with vigorous stirring. The flask was evacuated and kept under vacuum for 1 hr. 1.44 mL (15.5 mmole) ethoxymethyl-chloride was added still at 0 °C. The mixture was further stirred for 1 hr at room temperature then it was evaporated in vacuo to dryness. The oily residue was extracted with ether, the ether phase was evaporated and the remaining solid material was crystallized twice from 70 mL iso-proppyl alcohol. Yield 1.7g (58%) **144**.

146 mg (0.5 mmole) **144** was reduced, coupled with 1-H-indole-2,5-dicarboxylic acid dipentafluorophenyl ester (Example 1, Step B), reacted with ethylenediamine and purified as described for **106** in Example 53. Yield 40 mg (55%) B507-b. ES MS: 722.49 (calcd. for $M+H^+$: 722.34).

EXAMPLE 78

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-methoxyethoxymethyl-1H-indol-5-yl]}-amide}, **145**

Compound 145 was synthesized as generally described for **142** in Example 77 using methoxyethoxymethyl chloride. Yield 22 mg (27%). ES MS: 782.52 (calcd. for $M+H^+$: 782.36).

EXAMPLE 79

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-amino-ethylcarbamoyl)-1-methoxymethyl-1H-indol-5-yl]}-amide}, **146**

Compound **146** was synthesized as described for **142** in Example 77 using methoxymethyl chloride. Yield 20.3 mg (29%). ES MS: 694.35 (calcd. for $M+H^+$: 694.31).

EXAMPLE 80

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-alanyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **147**

To a solution of 25 mg (0.031 mmole) of **106** (Example 53) in DMF 21.5 mg (0.075 mmole) Boc-Ala-Opfp (**148**) and 22 μL (0.124 mmole) DIEA was added and the mixture was stirred at room temperature for 2 hrs. It was evaporated to dryness, triturated with ether and dried. The tBoc protecting group was removed by dissolving the dried solid material in TFA containing 20% anisol and reacting for 30 minutes. The crude product was precipitated with ether, washed 3 times with ether and was dried. It was purified with HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 0% to 60% acetonitrile gradient over 30 minutes, flow 20 mL/min). The purified compound was converted to HCl salt by dissolving in 2 mL methanol, treating with 1 mL 4N HCl in dioxane and precipitating with ether to yield 16 mg (68 %) **147**. ES MS: 748.31 (calcd. for $M+H^+$: 748.33).

EXAMPLE 81

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-phenylalanyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **149**

Compound **149** was synthesized as described for Compound **147** in Example 80, using Fmoc-Phe-Opfp (**150**), except the Fmoc protecting group was removed by treatment of the triturated and dried material with 20% piperidine in DMF for 30 minutes at room temperature. The piperidine reagent was evaporated and the remaining oil was triturated with ether. The solid crude product was purified and converted to HCl salt as described above in Example 80. Yield 19.2mg (68%) **149**; MS: 900.37 (calcd. for $M+H^+$: 900.40).

EXAMPLE 82

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-leucyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **151**

Compound **151** was synthesized as described for Compound **149** in Example 81, using Fmoc-Leu-OPfp. Yield 15.4mg (59%) **151**; MS: 832.41 (calcd. for $M+H^+$: 832.43).

EXAMPLE 83

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-isoleucyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **152**

Compound **152** was synthesized as described for Compound **149** in Example 81, using Fmoc-Ile-OPfp. Yield 13.2mg (50%) **152**; MS: 832.41 (calcd. for $M+H^+$: 832.43).

EXAMPLE 84

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-valyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **153**

Compound **153** was synthesized as described for Compound **149** in Example 81, using Fmoc-Val-OPfp. Yield 17.1mg (68%) **153**; MS: 804.39 (calcd. for $M+H^+$: 804.40).

EXAMPLE 85

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-glycyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **154**

Compound **154** was synthesized as described for Compound 149 in Example 81, using Fmoc-Gly-OPfp. Yield 18.5mg (82%) **154**; MS: 720.29 (calcd. for $M+H^+$: 720.30).

EXAMPLE 86

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-glutamyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **155**

Compound **155** was synthesized as described for Compound **149** in Example 81, using Fmoc-Glu(OtBu)-OPfp. The OtBu protecting group was removed as described for the removing of tBoc group in Example 80. Yield 3.2mg (11%) **155**; MS: 864.37 (calcd. for $M+H^+$: 864.35).

EXAMPLE 87

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-ornithyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **156**

Compound **156** was synthesized as described for Compound **155** in Example 86, using Fmoc-Orn(Boc)-OPfp. Yield 18.4mg (71%) **156**; MS: 834.42 (calcd. for $M+H^+$: 834.41).

EXAMPLE 88

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(N-acetyl- gamma- L-glutamyl)-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **157**

Compound **157**was synthesized as described for Compound **147** in Example 80, using Boc-Glu(OSu)-OBzl, except the Bzl and tBoc protecting groups were removed by treatment of the triturated and dried material with a mixture of 500 μL thioanisol, 250μL EDT 5 mL TFA and 500 μL TFMSA for 2 hrs at room temperature. The crude product was precipitated with ether, purified and converted to HCl salt as described in 80. Yield 6.8mg (25%) **157**; MS: 472.67 (calcd. for $M+2H^+$: 472.67).

EXAMPLE 89

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-norleucyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **158**

Compound **158** was synthesized as described for Compound **149** in Example 81, using Fmoc-Nle-OPfp. Yield 15.3mg (61%) **158**; MS: 832.41 (calcd. for $M+H^+$: 832.43).

EXAMPLE 90

Synthesis of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-lysyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **159**

Compound **159** was synthesized as described for Compound **147** in Example 80, using Boc-Lys(Boc)-OSu. Yield 19 mg (73%) **159**; MS: 862.45 (calcd. for $M+H^+$: 862.45).

EXAMPLE 91

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(L-2,3-diaminopropyl)-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **160**

51 mg (0.093 mmole) Fmoc-Dap(Fmoc)-OH was dissolved in DMF. 22 μL (0.124 mmole) DIEA was added followed by 15 μL (0.087 mmole) TFA-Opfp and the mixture was stirred for 15 minutes at room temperature. This activated acid solution was used to synthesize **160** as described for Compound **149** in Example 81. Yield 11.9 (49%) **160**; MS: 778.37 (calcd. for $M+H^+$: 778.356).

EXAMPLE 92

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(L-2,4-diaminobutyryl)-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **161**

Compound **161** was synthesized as described for Compound **160** in Example 91, using Fmoc-Dab(Fmoc)-OH. Yield 9.3 mg (38%) **161**; MS: 805.39 (calcd. for $M+H^+$: 805.39).

EXAMPLE 93

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(N-methyl-L-valyl)-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **162**

Compound **162** was synthesized as described for Compound **160** in Example 91, using Fmoc-MeVal-OH. Yield 19.1 mg (76%) **162**; MS: 832.42 (calcd. for $M+H^+$: 832.43).

EXAMPLE 94

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-arginyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **163**

Compound **163** was synthesized as described for Compound **157** in Example 88, using Boc-Arg(Z_2)-OSu. Yield 23.3 mg (84%) **163**; MS: 918.45 (calcd. for $M+H^+$: 918.46).

EXAMPLE 95

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(L-2,3-diaminopropyl)-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **164**

Compound 164 was synthesized as described for Compound **160** in Example 91. Yield 12.9 mg (55%) **164**; MS: 778.35 (calcd. for $M+H^+$: 778.36).

EXAMPLE 96

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(L-2,4-diaminobutyryl)-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **165**

Compound **165** was synthesized as described for Compound **161** in Example 92. Yield 11.2 mg (46%) **165**; MS: 805.39 (calcd. for $M+H^+$: 805.39).

EXAMPLE 97

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-(N-methyl-L-valyl)-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **166**

Compound **166** was synthesized as described for Compound **162** in Example 93. Yield 12.7 mg (50%) **166**; MS: 832.42 (calcd. for $M+H^+$: 832.43).

EXAMPLE 98

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-threonyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **167**

Compound **167** was synthesized as described for Compound **147** in Example 80, using Boc-Thr-OSu. Yield 20.4 mg (84%) **167**; MS: 808.37 (calcd. for $M+H^+$: 808.36).

EXAMPLE 99

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-threonyl-amido-ethylcarbamoyl)-1H-indol-6-yl]-amide}, **168**

Compound **168** was synthesized as described for Compound **147** in Example 80, using Boc-Thr-OSu. Yield 18.8 mg (77%) **168**; MS: 808.37 (calcd. for $M+H^+$: 808.36).

EXAMPLE 100

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-glycyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **169**

Compound **169** was synthesized as described for Compound **147** in Example 80, using Boc-Gly-OSu. Yield 13.2 mg (73%) **169**; MS: 720.28 (calcd. for $M+H^+$: 720.30).

EXAMPLE 101

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-acetamino-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **170**

Compound **170** was synthesized as described for Compound **147** in Example 80, using acetic anhydride, except no protecting group removal was necessary. Yield 11 mg (60%) **170**; MS: 690.16 (calcd. for $M+H^+$: 690.28).

EXAMPLE 102

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-glutamyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **171**

Compound **171** was synthesized as described for Compound **149** in Example 81, using Fmoc-Glu(OtBu)-OPfp. The OtBu protecting group was removed as described for the removing of tBoc group in Example 80. Yield 5mg (23%) **171**; MS: 864.37 (calcd. for $M+H^+$: 864.35).

EXAMPLE 103

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-lysyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **172**

Compound **172** was synthesized as described for Compound **147** in Example 80, using Boc-Lys(Boc)-OSu. Yield 19 mg (88%) **172**; MS: 862.45 (calcd. for $M+H^+$: 862.45).

EXAMPLE 104

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-valyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **173**

Compound **173** was synthesized as described for Compound **149** in Example 81, using Fmoc-Val-OPfp. Yield 15.4mg (76%) **173**; MS: 804.41 (calcd. for $M+H^+$: 804.40).

EXAMPLE 105

Synthesis of of 1H-Indole-2,5-dicarboxylic acid bis-{[2-(2-L-aspartyl-amido-ethylcarbamoyl)-1H-indol-5-yl]-amide}, **174**

Compound **174** was synthesized as described for Compound **149** in Example 81, using Fmoc-Asp(OtBu)-OPfp. The OtBu protecting group was removed as described for the removing of tBoc group in Example 80. Yield 9.3 mg (44%) **174**; MS: 836.38 (calcd. for $M+H^+$: 836.31).

EXAMPLE 106

Synthesis of Boc-Py-HBA-AMPS (**175**)

Boc Py (5mmol, 1.20 g) was dissolved in 20 mL dichloromethane and 0.774mL (5 mmol) DIC, 100 mg (0.8mmol) DMAP were added. This solution was added to 2.5g of Hba-AMPS resin and agitated overnight at room temperature. After filtering the solution off, the resin was washed three times with DMF, three times with dichloromethane, 2 times with methanol and two times with diethyl ether. Each washing volume was approximately equivalent to the volume of the resin. The resin was subsequently dried under high vacuum and weighed. Yield of **175**: 2.9226g corresponding to a substitution of 0.76 mmol/g.

EXAMPLE 107

Synthesis of Boc-5-Ain-HBA-AMPS (**176**)

Boc-5-Ain (5mmol, 1.38 g) was dissolved in 20 mL DMF and 2.21g (2 eq.) BOP, 0.871 mL (2 eq.) DIEA were added. This solution was added to 2.5g of Hba-AMPS resin and agitated overnight at room temperature. After filtering the solution off, the resin was washed three times with DMF, three times with dichloromethane, 2 times with methanol and two times with diethyl ether. Each washing volume was approximately equivalent to the volume of the resin. The resin was subsequently dried under high vacuum and weighed. Yield of (**176**): 2.9327g corresponding to a substitution of 0.626 mmol/g.

EXAMPLE 108

Exemplary Synthesis Procedure for Compound (**177**) (*Scheme 7*)

0.03 mM resin **176** was washed three times with ca. 5 mL DMF and three times with ca. 5 mL dichloromethane. The swelled resin was then washed for 1 minute with a mixture of 25% trifluoroacetic acid/2% anisole in dichloromethane and after draining treated for another 20 minutes with the same mixture. After draining, the resin was washed two times with ca. 5 mL dichloromethane and two times with ca. 5 mL DMF to give unprotected **178**. Dipeptide **179** (34.4mg, 0.09mmol), synthesized separately in solution, was dissolved in 2 mL DMF and mixed with 32.4 mg HBTU and 30.9 μL DIEA. After 5 min this mixture was added to resin and agitated for 2 hours to give resin-bound tripeptide **180**. This resin was treated for 2 hours with 2 mL neat ethylenediamine to give product **177**. The resin was filtered off, the solution is

evaporated *in vacuo* and the resulting oil was dissolved in methanol and precipitated with diethyl ether. The resulting precipitate was spun down, the ether decanted and the product dried *in vacuo*. This crude product was HPLC-purified (Vydac 12 µm, C18 2.2x25 cm column, 0% to 80% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified **177** (see table 1).

Compounds **181-188** were synthesized using the same synthesis procedure as above, but with the following modifications: Compounds **181**, **182**, **183**, **184**, and **188** started their synthesis with resin **175**, compounds **185**, **186**, and **187** used resin **176**. The amines used, to cleave tripeptide precursors from the resins to form compounds **181** through **188** are listed in table 1 under "Amines Used." All amines were used neat (2mL each), except for 1,4-diamino butane, which was dissolved in 600µL tetrahydofuran.

Table 1

Compound Number	Resin Used	Amine Used	Yield in mg	MS found ($M+H^+$)	MS calculated ($M+H^+$)
181	175	1,4-diamino butane	7.7	575.35	575.69
182	175	Ethylene diamine	5.4	547.30	547.64
183	175	DP	8.5	589.35	589.72
184	175	DE	9.5	575.33	575.69
185	176	1,4-diamino butane	8.3	611.33	611.72
177	176	Ethylene diamine	8.6	583.30	583.67
186	176	DP	13.1	625.35	625.75
187	176	DE	14.7	611.33	611.72
188	175	Ethanolamine	21.1	548.27	548.62

EXAMPLE 109

Exemplary Synthesis Procedure for compound **191** *(Scheme 8)*

0.03 mM resin **176** was washed three times with ca. 5 mL DMF and three times with ca. 5 mL dichloromethane. The swelled resin was then washed for 1 minute with a mixture of 25% trifluoroacetic acid/2% anisole in dichloromethane and after draining treated for another 20 minutes with the same mixture. After draining, the resin was washed two times with ca. 5 mL dichloromethane and two times with ca. 5 mL DMF to give unprotected **178**. Dipeptide **189** (42.2mg, 0.09mmol), synthesized separately in solution, was dissolved in 2 mL DMF and mixed with 32.4 mg HBTU and 30.9 µL DIEA. After 5 min this mixture was added to resin

and agitated for 2 hours to give resin-bound tripeptide **190**. This resin was treated for 2 hours with 2 mL of 2M methylamine in THF to give product **191**. The resin was filtered off, the solution was evaporated *in vacuo* and the resulting oil was dissolved 500μL anisol and 2 mL TFA. After 30 min stirring, this solution was evaporated *in vacuo*, dissolved in methanol and precipitated with diethyl ether. The resulting precipitate was spun down, the ether decanted and the product dried *in vacuo*. This crude product was HPLC-purified (Vydac 12 μm, C18 2.2x25 cm column, 0% to 80% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified **191** (see table 2).

Compounds **192-200** were synthesized using the same synthesis procedure as above, but with the following modifications: Compounds **192, 193, 194, 195,** and **200** started their synthesis with resin **175**, compounds **196, 197, 198,** and **199** used resin **176**. The amines used, to cleave tripeptide precursors from the resins to form compounds **192** through **200** are listed in table 2 under "Amines Used." Ethylene diamine was used neat (2mL), all other amines were used in solution: Methyl amine (2M in THF), 1,4-diamino butane (2mL dissolved in 600μL tetrahydofuran), diethylenetriamine (10 eq. in 2 mL THF), N,N'-Bis(3-aminopropyl)-1,3-propanediamine (10eq. in 2mL THF), and Tris(2-aminoethyl)amine (10eq. in 2mL THF).

Table 2

Compound Number	Resin Used	Amine Used	Yield in mg	MS found ($M+H^+$)	MS calculated ($M+H^+$)
192	175	Methyl amine	14.3	505.25	505.56
193	175	1,4-diamino butane	25.9	562.30	562.65
194	175	Diethylenetriamine	4.1	577.31	577.67
195	175	N,N'-Bis(3-aminopropyl)-1,3-propanediamine	8.7	662.41	662.82
191	176	Methyl amine	17.5	541.25	541.59
196	176	1,4-diamino butane	26.1	598.31	598.69
197	176	Diethylenetriamine	8.5	613.32	613.70
198	176	Ethylene diamine	27.9	570.28	570.63
199	176	Tris(2-aminoethyl)amine	13.0	620.37	620.74
200	175	Tris(2-aminoethyl)amine	24.4	656.36	656.77

EXAMPLE 110

Exemplary Synthesis Procedure for compound **201** *(Scheme 9)*

0.05 mM resin **175** was washed three times with ca. 5 mL DMF and three times with ca. 5 mL dichloromethane. The swelled resin was then washed for 1 minute with a mixture of 25% trifluoroacetic acid/2% anisole in dichloromethane and after draining treated for another 20 minutes with the same mixture. After draining, the resin was washed two times with ca. 5 mL dichloromethane and two times with ca. 5 mL DMF to give unprotected **178**. Dipeptide **202** (61.1mg, 0.10mmol), synthesized separately in solution, was dissolved in 2 mL DMF and mixed with 36.1 mg HBTU and 34.7 µL DIEA. After 5 min this mixture was added to resin and agitated for 2 hours to give resin-bound tripeptide **203**. This resin was treated for 2 hours with 2 mL of neat ethylenediamine to give product **201**. The resin was filtered off, the solution was evaporated *in vacuo* and the resulting oil was dissolved 500µL anisol and 2 mL TFA. After 30 min stirring, this solution was evaporated *in vacuo*, dissolved in methanol and precipitated with diethyl ether. The resulting precipitate was spun down, the ether decanted and the product dried *in vacuo*. This crude product was HPLC-purified (Vydac 12 µm, C18 2.2x25 cm column, 0% to 80% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified **201** (see table 3).

Compounds **204-210** were synthesized using the same synthesis procedure as above, but with the following modifications: Compounds **204**, **205**, **206**, and **207** started their synthesis with resin **175**, compounds **208**, **209** and **210** used resin **176**. The amines used, to cleave tripeptide precursors from the resins to form compounds **201** through **210** are listed in table 3 under "Amines Used." Ethylene diamine and butyl amine were used neat (2mL), all other amines were used in solution: Methyl amine (2M in THF), octyl amine (1mL dissolved in 1 mL tetrahydofuran), and 2-methylaminopyridine (1mL dissolved in 1 mL tetrahydofuran).

Table 3

Compound Number	Resin Used	Amine Used	Yield in mg	MS found ($M+H^+$)	MS calculated ($M+H^+$)
204	175	Methyl amine	8.5	547.25	547.59
205	175	butyl amine	17.1	589.30	589.67
206	175	Octylamine	8.1	645.37	645.78
207	175	Ethylene diamine	12.6	576.28	576.63
208	176	Octylamine	20.0	681.36	681.81

209	176	butyl amine	10.4	625.31	625.70
210	176	Ethylene diamine	12	612.29	612.66
211	176	2-methyl aminopyridine	4.8	660.29	660.71

EXAMPLE 111

Exemplary Synthesis Procedure for compound **211** *(Scheme 10)*

1-Methyl-4-nitro-imidazole-2-carboxylic acid ethyl ester (4g, 20mmol) were put into a screw cap flask and overlayered with 20 mL ethylene diamine and then placed overnight into a 55°C oven. The solvent was evaporated *in vacuo* and subsequently dried under high vacuum to give **212**.

212 was dissolved in 100 mL DMF and 6.55g di-*tert*-butyl dicarbonate were added portionwise to the solution. After 1 hr stirring, the reaction was concentrated to 50 mL and separated between chloroform (150 mL) and 0.5 M sodium bicarbonate (150 mL). The organic layer was washed twice with 0.1M sulfuric acid, twice with water, dried over anhydrous sodium sulfate to give a yellow oil that later solidified. Recrystallisation with hot toluene gave 3.51g (56% overall yield) of **213**. ^{1}H-NMR (DMSO-d_6) 8.74 (tr, 1H, δ=5.9, NH), 8.54 (s, 1H, imidazole C-H), 6.87 (tr, 1H, δ=5.2, NH), 3.99 (s, 3H, Me), 3.23-3.28 (m, 2H, CH_2), 3.04 (q, 2H, δ=5.9, CH_2), 1.35 (s, 9H, tBu); m.p. 138-139°C.

213 (3.12g, 10 mmol) was dissolved under heating in 100 mL ethyl acetate. Methanol (20 mL), followed by 1 g of 5% palladium on carbon were added, and the hydrogenation was started in a Parr shaker at 37 psi. After 30 min. the pressure stabilized and the reaction was stopped. The catalyst was filtered off and the solvent was evaporated in vacuo. Drying under high vacuum gave a yellow/brown oil **214**.

214 was dissolved in 15 mL DMF, 3.59g (9 mmol) Pfp-ester SL40, which was previously synthesized in solution, was added and the reaction flask put into a 55°C oven. After an overnight reaction the TLC indicated an incomplete reaction. SL38 (0.7g) were hydrogenated as described above and its reaction product (**214**) was dissolved in 2 mL DMF and added to the solution. After continuing the reaction for another day at 55°C the reaction was evaporated and the resulting brown oil purified via silica gel colomn cromatography. Increasing the gradient slowly from 9:1 to 1:1 tolene /ethyl acetate 320 mg (8%)of product **215** were obtained; ^{1}H-NMR (DMSO-d_6) 12.12 (s, 1H, NH), 10.61 (s, 1H, NH), 8.38 (s, 1H, indole C4-H), 7.97 (tr, 1H, NH, δ=5.4), 7.89 (d, 1H, indole C6-H, δ=8.6), 7.55 (s, 1H,

imidazole C5-H), 7.48 (d, 1H, indole C7-H, δ=8.7), 7.35 (s, 1H, indole C3-H), 6.89 (tr, 1H, NH, δ=4.8), 4.34 (q, 2H, O-CH_2, δ=6.9), 3.94 (s, 3H, CH_3), 3.31 (CH_2 signal under H_2O), 3.06 (q, 2H, CH_2, δ=6.0), 1.348 (m, 12 H, *tert*-Bu, CH_3) ESI-MS: mass calculated ($M+H^+$) 499.23, found 499.22.

215 (300 mg) were dissolved in 4 mL of methanol and heated to 60°C. 1.2 mL of 1N aqueous sodium hydroxide solution were added and the reaction was stirred at 60°C for three hours. The reaction mixture was subsequently evaporated and redissolved in 5 mL of water. Acidification with 1 N aqueous hydrochloride to pH3 precipitated the product, which was spun down. Four washings with water (30 mL each) brought the pH to 4.5.The resulting crystals were lyophilized and dried under high vacuum over P_2O_5 to give 248.9 mg (88%)of **216.**

0.05 mM resin **176** was washed three times with ca. 5 mL DMF and three times with ca. 5 mL dichloromethane. The swelled resin was then washed for 1 minute with a mixture of 25% trifluoroacetic acid/2% anisole in dichloromethane and after draining treated for another 20 minutes with the same mixture. After draining, the resin was washed two times with ca. 5 mL dichloromethane and two times with ca. 5 mL DMF to give unprotected **178**. Dipeptide **216** (61.1mg, 0.10mmol), synthesized as described above, was dissolved in 2 mL DMF and mixed with 36.1 mg HBTU and 34.7 µL DIEA. After 5 min this mixture was added to resin and agitated for 2 hours to give resin-bound tripeptide **217**. This resin was treated for 2 hours with 2 mL of neat ethylenediamine. The resin was filtered off, the solution was evaporated *in vacuo* and the resulting oil was dissolved 500µL anisol and 2 mL TFA. After 30 min stirring, this solution was evaporated *in vacuo*, and stirred for 2 hours with 0.5 mM *N,N'*-Bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine dissolved in 2 mL DMF. The solution was evaporated *in vacuo* and the resulting oil was dissolved 500µL anisol and 2 mL TFA. After 30 min stirring, this solution was evaporated *in vacuo*, dissolved in methanol and precipitated with diethyl ether. The resulting precipitate was spun down, the ether decanted and the product dried *in vacuo*. This crude product was HPLC-purified (Vydac 12 µm, C18 2.2x25 cm column, 0% to 80% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified **211** (see table 4).

Table 4

Compound	Resin	Amine Used	Yield	MS found	MS

Number	Used		in mg	$(M+2H^+)/2$	calculated $(M+2H^+)/2$
218	175	ethylene diamine	19.1	310.16	310.34
211	176	ethylene diamine	22.1	328.16	328.35

EXAMPLE 112

Exemplary Synthesis Procedure for compound **219** *(Scheme 11)*

2,2-Bis(azidomethyl)-1,3-propanediol was synthesized from 2,2-Bis(bromomethyl)-1,3-propanediol in two steps, similarly to a procedure published previously (J. Med. Chem. 1989, 32, 2015-2020).

2,2-Bis(bromomethyl)-1,3-propanediol (3g, 11.453 mmol) was stirred with 3g (4 eq.) of sodium azide in 100 mL DMF at 120°C for 2 days. The reaction was cooled to room temperature, filtered, evaporated to ca 10mL. The residue was taken up in 100 mL dichloromethane, filtered and again evaporated. The residue was checked by NMR, which contained only DMF and 2,2-bis(azidomethyl)-1,3-propanediol. product was not further evaporated, but used in the next step. ^{1}H-NMR (DMSO-d_6) 4.73 (tr, 2H, OH), 3.28 (s, 4H, 2*CH_2), 3.25 (d, 4H, 4.1 Hz); ^{13}C-NMR (DMSO-d_6) 60.43, 51.80, 46.20.

2,2-Bis(azidomethyl)-1,3-propanediol was dissolved in 20 mL ethanol, cooled to 0°C and 500 mg of 5% Pd/$CaCO_3$ were added. After bubbling Ar through the mixture for 15 min, the mixture was hydrogenated for 6 hr by bubbling H_2 through the suspension. The brown suspension turned black after 1-2 hours. Filtration, evaporation and drying yielded greasy 1.5g of crystals. The crude product (2,2-bis(aminomethyl)-1,3-propanediol was used without further purification. ^{1}H-NMR (DMSO-d_6) 4.22 (s, 4H, 2*CH_2), 2.69 (br s, 6H, 2*OH, 2*NH_2), 2.47 (s, 4H, 2*CH_2); ^{13}C-NMR (DMSO-d_6) 59.41, 39.57, 39.19; ESI-MS: : mass calculated $(M+H^+)$ 135.11, found 135.12.

220 was synthesized from **221** in three steps. **221** (6g, 25.7 mmol) was suspended in 125 mL methanol and heated to 55°C. 3N aq. Sodium hydroxide solution was added, whereupon all of the starting material dissolved. After stirring for 5 hours at 55°C, the solution was acidified to pH 2, and filtered. The filtrate was washed with water (50mL) and subsequently dried over phosphorus pentoxide to give indole-2,5-dicarboxylic acid **222** in quantitative yield. ^{1}H-NMR (DMSO-d_6): 12.06 (s, 1H, NH), 8.34 (s, 1H, CH), 7.82 (d, 1H, CH, δ=8.8 Hz), 7.47 (d, 1H, CH, δ=8.8 Hz), 7.23 (s, 1H, CH); m.p. 314-315°C.

Indole-2,5-dicarboxylic acid (**222**) (3g, 12.86 mmol) was dissolved in 40 mL DMF. Diisopropylethylamine (5.37mL, 2.4 eq) and 5.3 mL (2.4 eq) of pentafluorophenol trifluoro acetate were added to the reaction mixture. The reaction mixture was stirred overnight, evaporated, and separated between 150mL ethyl acetate and 150mL satured aq. sodium bicarbonate solution. The aqueous layer was extracted two more times with ethyl acetate (150 mL each); the organic layers were combined and dried over anhydrous sodium sulfate. The crude material was loaded on a silica gel-filled Büchner funnel and the product was eluted with 50% hexane/toluene mixture. 2.13 g (30.8%) of product **223** was obtained.

5-Nitro indole-2-carboxylic acid ethyl ester (654 mg, 2.79 mmol) was hydrogenated using 5% Pd/C (0.5 g) as a catalyst at 30 psi pressure for 30 min. Filtration through a frit to remove the catalyst, evaporation in vacuo and drying under high vacuum yielded free amine (**224**). It was immediately dissolved in 3 mL DMF and 500mg **223** were added. The reaction was kept at 55°C overnight and then evaporated. The crude material was recrystallized from hot ethanol to give 273 mg (50.8%) of product (**220**) after evaporation and drying. ^{1}H-NMR (DMSO-d_6): 11.98 (s, 1H, NH-indole), 11.86 (s, 1H, NH-indole), 11.81 (s, 1H, NH-indole), 10.25 (s, 1H, CONH), 10.11 (s, 1H, CONH), 8.39 (s, 1H, CH), 8.15 (s, 1H, CH), 7.86 (dd, 1 H, CH, δ=9.2, δ=1.4), 7.53-7.62 (m, 4H, 4*CH), 7.43 (tr, 2H, δ=9.1), 7.15 (d, 2 H, 2*CH, δ=7.9), 4.33 (q, 4 H, 2*CH_2, δ=7.0), 1.34 (tr, 6H, 2*CH_3, δ=7.0).

30 mg of tripeptide (**220**) and 150 mg of diamine ((2,2-bis(aminomethyl)-1,3-propanediol) were dissolved in 1 mL DMF. The reaction was stirred for 3 days at room temperature and then 4 days at 55°C. Evaporation was followed by HPLC-purification (Vydac 12 µm, C18 2.2x25 cm column, 30% to 80% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified product. After lyophilisation, the product was dissolved in ice-cold methanol, acidified with 200 µL 4 N HCl/dioxane and then precipitated with diethyl ether. The product was centrifuged, the ether decanted. The final product was dried in vacuo to give purified **219**. In the synthesis of **225** 30 mg of tripeptide (**220**) were dissolved in 500 µL neat 2,2- Dimethyl-1,3-propanediamine. The same reaction conditions and purification procedure were chosen as for **219**.

Table 5

Compound Number	Amine Used	Yield in mg	MS found ($M+H^+$)	MS calculated ($M+H^+$)

219	(2,2-bis(aminomethyl)-1,3-propanediol	1.7	754.36	754.36
225	(2,2-dimethyl)-1.3-propane diamine	4.7	690.35	690.35

EXAMPLE 113

Exemplary Synthesis Procedure for compound **226** *(Scheme 12)*

227 (30mg) was dissolved in 2 mL DMF and brought to -20°C in an acetone/CO_2 bath. Diisopropyl ethylamine (19 µL, 2.2 eq) and 4-nitrobenzylchloroformate (24 mg) were added. After stirring at -20°C for 30 min., the reaction was stirred at room temperature overnight. Evaporation was followed by HPLC-purification (Vydac 12 µm, C18 2.2x25 cm column, 0% to 100% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min) to give purified compound. After lyophilisation, the product was dissolved in ice-cold methanol, acidified with 200 µL 4 N HCl/dioxane and then precipitated with diethyl ether. The product was centrifuged, the ether decanted. The final product was dried in vacuo to give purified **226** (see table 6). The same synthesis was performed with 4-methoxyphenyl chloroformate to give **228**.

Table 6

Compound Number	Chloroformate used:	Yield in mg	MS found (MH^+)	MS calculated (MH^+)
228	4-nitrobenzyl chloroformate	7.6	785.40	785.27
226	4-methoxyphenyl chloroformate	9.8	756.41	756.28

EXAMPLE 114

Synthesis Procedure for compound **229** *and* **230** *(Scheme 13)*

Benzimidazole **231** (349.5 mg, 1.2 mmol) were dissolved in pure TFA (5 mL) and left standing at room temperature for 30 min. Toluene was subsequently added and the solution evaporated in vacuo. This procedure was repeated twice. The resulting amine **232** was dried under high vacuum. **232** was then dissolved in 5 mL DMF and **223** (214.9 mg, 0.4 mmol) as

well as 6 eq. of diisopropylethylamine (418 μL) were added. This mixture was stirred for 1 week at room temperature. The reaction was monitored via HPLC purified (Vydac 12 μm, C18 2.2x25 cm column, 0% to 100% aqueous acetonitrile gradient over 20 min, flow rate 20 mL/min). Disappearance of a peak at 100% acetonitrile (corresponding to **223**) and appearance of a major peak at ca. 75% acetonitrile. The peak was very broad and consisted predominantly of dipeptide **233** as well as a minor amount of **234**. The substitution for **233** was assumed to be at the C-2 carboxy group, in accordance with several previous studies, which showed preferred substitution at this site. 30 mg of the mixture **233** and **234** were dissolved in 5 mL ethylenediamine and stirred for 1.5 days. The mixture was subsequently evaporated and HPLC-purified. Tripeptide was isolated and converted to the HCl salt, yielding 2.3 mg of final product (**229**).

235 (40 mg, 115 μmol) was dissolved in methanol/ ethyl acetate and hydrogenated for 30 min in a Parr Shaker at ca. 30 psi. The catalyst was filtered off, the solvent evaporated and the resulting free amine (SL61) dried under high vacuum. **236** was dissolved in 3 mL DMF and 30 mg (55.1 μmol) SL57 and 20μL (115 μmol) disopropylethyl amine were added. The reaction was stirred for 1 ½ days and then evaporated to give crude **237**. It was immediately dissolved in 200 μL anisol and 1800 μL TFA, left standing for 300 min, precipitated with ether, centrifuged, the ether decanted and subsequently dried. The compound was purified via preparative HPLC, lyophilized, dissolved in ice-cold methanol, acidified with 4 M HCl/dioxane, precipitated with ether, centrifuged, the ether was decanted and the product **230** dried in vacuo.

Table 7

Compound Number	Amine Used:	Yield in mg	MS found (MH^+)	MS calculated (MH^+)
229	EDA	2.3	608.32	608.25
230	-	5.1	579.17	579.21

EXAMPLE 115

Synthesis of pyridine-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-amide} **231**

Step A: 1-propyl-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester **232.**

4-Nitro-1H-pyrrole-2-carboxylic acid ethyl ester (5 g) was dissolved in 50 ml of dry EtOH, 50 ml of 1M sodium ethylate was added followed with 10 ml of CH_3I. The reaction mixture was heated at 80°C for 4 hours, cooled down to room temperature and distributed between water and chloroform. The organic phase was washed with water, dried with sodium sulfate and evaporated. The residue was recrystallized from hexane to yield 4.72 g (77%) of 1-propyl-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester **232**.

H^1-NMR (DMSO-d6): 0.79 (t, 3H, CH_3), 1.25 (t, 3H, CH_3), 1.68 (m, 2H, CH_2), 4.17-4.28 (m, 4H, $2CH_2$), 7.23 and 8.24 (d, 1H, pyrrole)

Step B: Synthesis of 1-(propyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amide **233**.

Compound **232** (5 g) was suspended in 30 ml of methanol, 2M NaOH (10 ml) was added, and the mixture was stirred at 50°C for 2 hours. The clear solution was diluted with water (50 ml) and 1N HCl was added dropwise to get pH2.5. The white residue was filtered, washed with water and dried to get 4.6g (95%) of the acid **234**. ES MS: 220.47 ($M+Na-H^+$). The acid **234** was suspended in $SOCl_2$ (20 ml) and the mixture was refluxed for 4 hours until clear solution was obtained. The reaction mixture was evaporated and dried by co-evaporation with toluene (10 ml x 3). The obtained chloroanhydride **235** was used without purification. Anhydride **235** was dissolved in toluene (10 ml) and 3-aminopropionitrille (3.9 ml, 54.3 mmol) was added. The mixture was kept for 1 hour at ambient temperature and evaporated. The white precipitate was suspended in 0.1 N HCl, filtered, washed with water and dried. Recrystallized from methanol yielded 4.4 g (81%) of **233**.

ES MS: 251.87 ($M+H^+$). H^1-NMR (DMSO-d6): 0.73-0.79 (m, 3H, CH_3), 1.63-1.70 (m, 2H, CH_2-propyl), 2.84-2.89 (m, 2H, CH_2-CN), 3.65-3.42 (m, 2H, CH_2-NH), 4.27-4.32 (m, 2H, CH_2-N), 7.43 and 8.15 (d, 1H, pyrrole), 8.83 (bt, 1H, NH).

Step C: Synthesis of 1-propyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amidine **236.**

The solution of 1-(propyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amidine **233** (2.5g, 10 mmol) in 50 ml of dry ethanol was cooled to 0-5°C and saturated with HCl gas. The mixture was sealed and refrigerated for 20 hours. The mixture was allowed to warm to room temperature and ethanol was evaporated . The solid was dissolved in 50 ml of

dry ethanol and saturated with ammonia gas. The sealed mixture was kept overnight at room temperature and evaporated. The solid was dissolved in 10 ml of methanol, and ether was added to precipitate 2.4 g (94%) of the target product **236** as a white solid. ES MS: 268.92 ($M+H^+$). H^1-NMR (DMSO-d6): 0.79 (t, 3H, CH_3), 1.64-1.71 (m, 2H, CH_2-propyl), 2.62-2.66 (m, 2H, CH_2-CN), 3.49-3.55 (m, 2H, $\underline{CH_2}$-NH), 4.28-4.33 (m, 2H, $C\underline{H_2}$-N), 7.54 and 8.15 (d, 1H, pyrrole), 8.83 (t, 1H, NH).

Step D: Synthesis of pyridine-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-amide} **231.**

To stirred solution of 1-propyl-4-nitro-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amidine **236** (70 mg, 0.15 mmol) in methanol (20 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). The flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 25-30 psi. The resultant suspension was stirred vigorously at 23^{0}C for 45 min. The suspended material was filtered, the filtrate was evaporated to dryness. The resulted 1-propyl-4-amino-1H-pyrrole-2-carboxylic acid (2-carbamimidoyl-ethyl)-amidine **237** was used for the next step without purification. The solution of freshly prepared **237** in 3 ml of dry DMF was added to pyridine-2,5-dicarboxylic acid dipentafluorophenyl ester (25 mg, 0.07 mmol), the reaction mixture was stirred for 15 hours at 55^{0}C, cooled down, and purified by HPLC (Vydac 12 μm C_{18} 2.2x25 cm column, 10-70% acetonitrile gradient over 40 min, flow 10 mL/min) to give pyridine-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-amide} **231** as a bis-trifluoroacetate salt: 33 mg (57%). ES MS: 606.71 ($M+H^+$). The bis-trifluoroacetate salt of **231** was dissolved in 2 ml of methanol saturated with HCl, 35 ml of diethyl ether was added, the precipitate of **231** as HCl salt was separated and dried.

EXAMPLE 116

Synthesis of N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-propyl-1H-pyrrol-3-yl]-isophthalamide **238.**

Compound **231** was synthesized as described for compound **231** above. Yield 52% of compound **231**. ES MS: 605.72 ($M+H^+$).

EXAMPLE 117

N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **239**

Step A: Synthesis of 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester **240.**

Compound **240** was synthesized as described in example 1, step A, using 1-bromo-3-methyl-butane as an alkylating agent. The yield is 6.5 g (94%). H^1-NMR (DMSO-d6): 0.87 (d, 6H, CH_3), 1.26 (t, 3H, CH_3), 1.49-1.62 (m, 3H, CH & CH_2), 1.68 (m, 2H, CH_2), 4.23 (q, 2H, CH_2), 4.33 (t, 2H, CH_2), 7.28 and 8.29 (d, 1H, pyrrole).

Step B: Synthesis of 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amide **241.**

Compound **241** was synthesized from ethyl carboxylate **240** as described in Example 115, step B. The yield is 5.1 g (83%). ES MS: 277.34 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.83-0.86 (m, 6H, CH_3), 1.40-1.51 (m, 1H, CH), 1.51-1.61 (m, 2H, CH_2-CH), 2.68-2.72 (m, 2H, CH_2 -CN), 3.35-3.42 (m, 2H, CH_2-NH), 4.33-4.37 (m, 2H, CH_2-N), 7.38 and 8.15 (d, 1H, pyrrole), 8.56 (bt, 1H, NH).

Step C: Synthesis of 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amidine **242.**

Compound **242** was synthesized from cyanoethylamide **241** as described in Example 115, step C in 10 mmol scale. The yield is 2.5 g (86%). ES MS: 295.34 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.88-0.86 (d, 6H, CH_3), 1.43-1.61 (m, 3H, CH & CH_2-CH), 2.61-2.65 (m, 2H, CH_2 -CN), 3.49-3.55 (m, 2H, CH_2-NH), 4.33-4.38 (m, 2H, CH_2-N), 7.51 and 8.18 (d, 1H, pyrrole), 8.73 (t, 1H, NH).

Step D: Synthesis of N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **239**

Compound **242** was condensed with dipentafluorophenyl ester of terephtalic acid as described in example 115, step D. Yield 47% of compound **239**. ES MS: 661.84 ($M+H^+$).

EXAMPLE 118

*Synthesis of Hexanedioic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}***243.**

Compound **243** was synthesized as described for compound **239** above (Example 117). Yield 52% of compound **243**. ES MS: 640.82 ($M+H^+$).

EXAMPLE 119

Cyclohexane-1,4-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **244.**

Compound **244** was synthesized as described for compound **239** above (Example 117). Yield 48% of compound **244**. ES MS: 667.87 ($M+H^+$).

EXAMPLE 120

*Biphenyl-4,4'-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}***245.**

Compound **245** was synthesized as described for compound **239** above (example 117). Yield 56% of compound **245**. ES MS: 737.98. ($M+H^+$).

EXAMPLE 121

Thiophene-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **246.**

Compound **246** was synthesized as described for compound **239** above (example 117). Yield 42% of compound **246**. ES MS: 666.81. ($M+H^+$).

EXAMPLE 122

N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-terephthalamide **247.**

Step A: Synthesis of 1-cyclopropylmethyl-4-nitro-1H-pyrrole-2-carboxylic acid ethyl ester **248.**

Compound **248** was synthesized as described in example 1, step A, using bromomethyl-cyclopropane as an alkylating agent. The yield is 4.8 g (74%). H^1-NMR (DMSO-d6): 0.37-0.42 & 0.65-0.72 (m, 2H, CH_2), 1.22-1.28 (m, 1H, CH), 1.37 (t, 3H, CH_3), 4.23 (d, 2H, CH_2), 4.32 (q, 2H, CH_2), 7.44 and 7.81 (d, 1H, pyrrole).

Step B: Synthesis of 1-(cyclopropylmethyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amide **249.**

Compound **249** was synthesized from ethyl carboxylate **248** as described in Example 115, step B. The yield is 4.3 g (78%). ES MS: 263.97 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.02-0.04 & 0.09-0.12 (m, 2H, CH_2), 0.89-1.00 (m, 1H, CH), 2.37-2.41(t, 2H, $C\underline{H_2}$-CN), 3.06-3.11 (dd, 2H, $\underline{CH_2}$-NH), 3.86-3.88 (d, 2H, , $C\underline{H_2}$-N), 7.09 and 7.87 (d, 1H, pyrrole), 8.45 (t, 1H, NH).

Step C: Synthesis of 1-(cyclopropylmethyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amidine **250.**

Compound **250** was synthesized from cyanoethylamide **249** as described in Example 115, step C in 10 mmol scale. The yield is 2.1 g (75%). ES MS: 280.01 ($M+ H^+$).

Step D: N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-terephthalamide **247.**

Compound **247** was synthesized as described for compound **231** in Example 115, step D. ES MS: 628.74 ($M+H^+$).

EXAMPLE 123

Pyridine-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-amide} **251.**

Compound **251** was synthesized as described for compound **247** above (Example 122). Yield 56% of compound **251**. ES MS: 630.73. ($M+H^+$).

EXAMPLE 124

N^1,N^4-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-2-nitro-terephthalamide **252**

Compound **252** was synthesized as described for compound **247** above (Example 122). Yield 54% of compound **252**. ES MS: 674.73. ($M+H^+$).

EXAMPLE 125

Thiophene-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-amide} **253**

Compound **253** was synthesized as described for compound **247** above (Example 122). Yield 41% of compound **253**. ES MS: 679.81. ($M+H^+$).

EXAMPLE 126

Pyrazine-2,5-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-amide} **254**

Compound **254** was synthesized as described for compound **247** above (Example 122). Yield 48% of compound **254**. ES MS: 630.71. ($M+H^+$).

EXAMPLE 127

Cyclohexa-1,3-diene-1,4-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)- 1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **255**

Compound **255** was synthesized as described for compound **239** above (Example 117). Yield 48% of compound **255**. ES MS: 663.85. ($M+H^+$).

EXAMPLE 128

1H-Pyrazole-3,5- dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)-1-cyclopropylmethyl-1H-pyrrol-3-yl]-amide} **256**

Compound **256** was synthesized as described for compound **247** above (Example 122).. Yield 48% of compound **256**. ES MS: 618.76. ($M+H^+$).

EXAMPLE 129

Cyclopropane-1,1-dicarboxylic acid bis-{[5-(2-carbamimidoyl-ethylcarbamoyl)- 1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **D21**

Compound **257** was synthesized as described for compound **239** above (Example 117). Yield 51% of compound **257**. ES MS: 625.79. ($M+H^+$).

EXAMPLE 130

N,N'-Bis-{1-(3-methyl-butyl)5-[2-(N-methylcarbamimidoyl)-ethylcarbamoyl)- -1H-pyrrol-3-yl]-terephthalamide **258**

Step A: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N-methylcarbamimidoyl)-ethyl]-amide **259**.

The solution of 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amidine **242** (0.5g) in 50 ml of dry ethanol was cooled to 0-5^{0}C and saturated with HCl gas. The mixture was sealed and refrigerated for 20 hours. The mixture was allowed to warm to room temperature and ethanol was evaporated . The solid was dissolved in 10 ml of dry ethanol and 1M solution of methylamine (3 ml) in methanol was added. The sealed mixture was kept overnight at 15^{0}C and evaporated. The solid was dissolved in 10 ml of methanol, and ether was added to precipitate 2.4 g (94%) of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N-methylcarbamimidoyl)-ethyl]-amide **259** as a white solid.
ES MS: 310.94 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.86-0.88 (d, 6H, CH_3-isopentyl), 1.43-1.61 (m, 3H, CH & $\underline{CH_2}$-CH), 2.60-2.65 (t, 2H, CH_2 $\underline{CH_2}$ -amidine), 3,37 (s, 3H, $\underline{CH_3}$-NH), 3.49-3.55 (m, 2H, $\underline{CH_2}$-NHCO), 4.33-4.38 (t, 2H, $C\underline{H_2}$-N), 7.51 and 8.18 (d, 1H, pyrrole), 8.73 (t, 1H, NHCO).

Step B: N,N'-Bis-[5-(2-carbamimidoyl-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **258**

Compound **258** was synthesized from **259** as described in Example115, step D. ES MS: 689.88. ($M+H^+$).

EXAMPLE 131

Pyridine-2,5-dicarboxylic acid bis-{[5-[2-(N-ethylcarbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **260**

Step A : Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N-ethylcarbamimidoyl)-ethyl]-amide **261**.

Compound **261** was synthesized from cyanoethylamide **242** as described in Example 130, step A, using ethylamine (3 ml). ES MS: 324.79 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.88-0.86 (d, 6H, CH_3 - isopentyl), 1.07-1.12 (t, 3H, CH_3-ethyl), 1.43-1.61 (m, 3H, CH & CH_2-CH), 2.52-2.56 (t, 2H, CH_2 CH_2 –amidine), 3.12-3.21 (m, 2H, CH_2 -ethyl), 3.49-3.55 (m, 2H, CH_2 CH_2 –amidine), 4.33-4.38 (t, 2H, CH_2-N), 7.39 and 8.20 (d, 1H, pyrrole), 8.54-8.60 (NH-amidine), 9.38 (t, 1H, NHCO).

Step B : Pyridine-2,5-dicarboxylic acid bis-{[5-[2-(N-ethylcarbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **262**

Compound **262** was synthesized as described in Example 115, step D. Yield 40% of compound **262**. ES MS: 718.92. ($M+H^+$).

EXAMPLE 132

N,N'-Bis-[5-[2-(N-isopropyl-carbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide

Step A : Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N-isopropylcarbamimidoyl)-ethyl]-amide **263**.

Compound **263** was synthesized from cyanoethylamide **242** as described in Example 130, step A, using isopropylamine (3 ml). ES MS: 338. 67 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.85-0.87 (d, 6H, CH_3- isopentyl), 1.09-1.11 (t, 3H, CH_3-isopropyl), 1.43-1.61 (m, 3H, CH & CH_2-CH), 2.59-2.64 (t, 2H, CH_2 CH_2 -amidine), 3.50-3.55 (m, 2H, CH_2 CH_2 –amidine), 3.73-3.80 (m, 1H, CH-isopropyl), 4.33-4.38 (t, , 2H, CH_2-N), 7.55 and 8.18 (d, 1H, pyrrole), 8.70 (t, 1H, NHCO).

Step B : N,N'-Bis-[5-[2-(N-isopropyl-carbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **264**

Compound **264** was synthesized as described in Example115, step D. ES MS: 745.98. ($M+H^+$).

EXAMPLE 133

Thiophene-2,5-dicarboxylic acid bis-[(1-(3-methyl-butyl)-5-{2-[N-(3-methyl-butyl)-carbamimidoyl]-ethylcarbamoyl}-1H-pyrrol-3-yl)-amide] **265**

Step A : Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid {2-[N-(3-methyl-butyl)isopropylcarbamimidoyl]-ethyl}-amide **266.**

Compound **266** was synthesized from cyanoethylamide **242** as described in Example 130, stepA, using 3-methyl-butylamine (3 ml). ES MS: 365. 27 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.79-0.81 (d, 6H, CH_3- isopentyl of pyrrole), 0.86-0.88 (d, 6H, CH_3- isopentyl of amidine), 1.31-1.60 (m, 6H, CH & CH_2-CH), 2.64-2.68 (t, 2H, CH_2 CH_2 -amidine), 3.12-3.17 (t, 2H, NH-CH_2 isopentyl of amidine), 3.50-3.55 (m, 2H, CH_2 CH_2 –amidine), 4.33-4.38 (t, , 2H, CH_2-N), 7.55 and 8.18 (d, 1H, pyrrole), 8.72 (t, 1H, NHCO).

Step B : Thiophene-2,5-dicarboxylic acid bis-[(1-(3-methyl-butyl)-5-{2-[N-(3-methyl-butyl)-carbamimidoyl]-ethylcarbamoyl}-1H-pyrrol-3-yl)-amide]

Compound **265** was synthesized as described in Example115, step D. Yield 54% of compound **265**. ES MS: 808.12. ($M+H^+$).

EXAMPLE 134

1H-Pyrazole-3,5-dicarboxylic acid bis-{[5-[2-(N-cyclopentylcarbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **267**

Step A: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N-cyclopentylcarbamimidoyl)-ethyl]-amide **268**

Compound **268** was synthesized from cyanoethylamide **242** as described in Example115, step D, using cyclopentylamine (3 ml). ES MS: 364. 37 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.85-0.87 (d, 6H, CH_3), 1.43-1.61 (m, 10H, CH & CH_2-CH of pyrrole and CH_2 of cycloppentyl), 1.82-1.88 (m, 2H, CH), 2.63-2.67 (t, 2H, CH_2 CH_2 -amidine), 3.47-3.56 (m,

2H, CH_2 $\underline{CH_2}$ –amidine), 4.33-4.38 (t, , 2H, $C\underline{H_2}$-N), 7.56 and 8.18 (d, 1H, pyrrole), 8.71 (t, 1H, NHCO).

Step B: 1H-Pyrazole-3,5-dicarboxylic acid bis-{[5-[2-(N-cyclopentylcarbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide} **267**

Compound **265** was synthesized as described in example115, step D. Yield 54% of compound **267**. ES MS: 788.03. ($M+H^+$).

EXAMPLE 135

N,N'-Bis-[5-[2-(N,N'-dimethyl-carbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **269**

Step A: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N, N'-dimethylcarbamimidoyl)-ethyl]-amide **270.**

The solution of 1-(3-methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid (2-cyano-ethyl)-amidine **242** (0.5g) in 50 ml of dry ethanol was cooled to 0-5^{0}C and saturated with HCl gas. The mixture was sealed and refrigerated for 20 hours. The mixture was allowed to warm to room temperature and ethanol was evaporated . The solid was dissolved in 10 ml of dry ethanol and 1M solution of methylamine (6 ml) in methanol was added. The sealed mixture was kept overnight at 55^{0}C and evaporated. The solid was dissoved in 10 ml of methanol, and ether was added to precipitate 2.4 g (94%) of the target product as a white solid. ES MS: 324.74 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.85-0.87 (d, 6H, CH_3-isopentyl), 1.43-1.61 (m, 3H, CH & $\underline{CH_2}$-CH), 2.76-2.79 (m, 5H, CH_2 $\underline{CH_2}$ –amidine and $\underline{CH_3}$-NH), 2.96-2.98 (d, 3H, $\underline{CH_3}$-NH), 3.46-3.55 (m, 2H, $\underline{CH_2}$-NHCO), 4.34-4.39 (t, 2H, $C\underline{H_2}$-N), 7.51 and 8.18 (d, 1H, pyrrole), 9.76 (t, 1H, NHCO).

Step B: N,N'-Bis-[5-[2-(N,N'-dimethyl-carbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **269**

Compound **269** was synthesized as described in Example115, step D. ES MS: 717.93 ($M+H^+$).

EXAMPLE 136

*Pyridine-2,5-dicarboxylic acid bis-{[5-[2-(N,N'-diethyl-carbamimidoyl)-ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}***271**

Step A: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(N,N'-diethylcarbamimidoyl)-ethyl]-amide **272**.

Compound **272** was synthesized from cyanoethylamide **242** as described above for compound **270** using ethylamine (3 ml). ES MS: 351.53. ($M+ H^+$). H^1-NMR (DMSO-d6): 0.86-0.88 (d, 6H, CH_3 - isopentyl), 1.07-1.17 (t, 6H, CH_3-ethyl), 1.42-1.60 (m, 3H, CH & CH_2-CH), 2.67-2.72 (t, 2H, CH_2 CH_2 –amidine), 3.12-3.21 (m, 2H, CH_2 -ethyl), 3.36-3.50 (m, 4H, CH_2 –NHCO and CH_2 -ethyl), 4.33-4.38 (t, 2H, CH_2-N), 7.39 and 8.20 (d, 1H, pyrrole), 8.63-8.71 (NH-amidine), 9.30 (t, 1H, NHCO).

Step B: Pyridine-2,5-dicarboxylic acid bis-{[5-[2-(N,N'-diethyl-carbamimidoyl)ethylcarbamoyl]-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-amide}**271**

Compound **271** was synthesized as described in Example115, step D. Yield 51% of compound **271**. ES MS: 775.03 ($M+H^+$).

EXAMPLE 137

N,N'-Bis-{1-(3-methyl-butyl)-5-[2-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-ethylcarbamoyl]-1H-pyrrol-3-yl}-terephthalamide **273**

Step A: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(1,4,5,6-tetrahydropyrimidin-2-yl)-ethyl]-amide **274.**

Compound **274** was synthesized from cyanoethylamide **242** as described above for compound **270** using 1,3-propylamine (6 ml). ES MS: 364. 37 ($M+ H^+$). H^1-NMR (DMSO-d6): 0.85-0.87 (d, 6H, CH_3), 1.42-1.61 (m, 3H, CH & CH_2-CH of pyrrole), 1.79-1.84 (m, 2H, CH_2 CH_2 CH_2), 2.55-2.60 (t, 2H, CH_2 CH_2 -amidine), 3.25 (m, 4H, CH_2 CH_2 CH_2), 3.40-3.52 (m, 2H, CH_2 –NHCO), 4.33-4.38 (t, 2H, CH_2-N), 7.48 and 8.18 (d, 1H, pyrrole), 8.15 and 9.78 (bs, 1H, NH-amidine), 8.89 (t, 1H, NHCO).

Step B: Synthesis of 1-(3-Methyl-butyl)-4-nitro-1H-pyrrole-2-carboxylic acid [2-(1,4,5,6-tetrahydropyrimidin-2-yl)-ethyl]-amide **273**

Compound **273** was synthesized as described in Example115, step D. ES MS: 741.96 ($M+H^+$).

EXAMPLE 138

N,N'-Bis-[5-(2-amino-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide

274

Step A: [2-({1-[1-(3-Methyl-butyl)-4-nitro-1H-pyrrol-2-yl]-methanoyl}-amino)-ethyl]-carbamic acid tert-butyl ester **275**

Compound **240** (1.3 g, 5 mmol) was dissolved in diethylamine (20 ml). This solution was kept for 50 hours at 60^{0}C and evaporated. The residue was dissolved in DMF (20 ml) and diBoc-carbonate (2.18 g, 10 mmol) was added. The reaction was kept 1 h at ambient temperature and evaporated. The residue was dissolved in chloroform (30 ml), washed with 0.1 M HCl (10x2 ml), 5% $NaHCO_3$ (10x2 ml), water, dried over sodium sulfate, and evaporated. The crude compound D33 was crystallized from toluene/hexane (4:1 v/v) to give white crystalls. The yield is 69% (1.27 g). H^1-NMR (DMSO-d6): 0.85-0.87 (d, 6H, CH_3), 1.34 (s, 9H, Boc), 1.42-1.61 (m, 3H, CH & CH_2-CH of pyrrole), 3.03-3.08 and 3.18-3.22 (each m, 2H, $NHCH_2CH_2NH$), 4.33-4.38 (t, 2H, CH_2-N), 6.85 (t, 1H, NHBoc), 7.48 and 8.18 (d, 1H, pyrrole), 8.36 (t, 1H, NHCO).

Step B: N,N'-Bis-[5-(2-amino-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **274**

To stirred solution of compound **275** (70 mg, 0.15 mmol) in methanol (20 ml) was added 10% Pd/C (Degussa type, Aldrich) (0.1 g). the flask was evacuated and then flushed 3 times with hydrogen and finally filled with hydrogen at 25-30 psi. The resultant suspention was stirred vigorously at 23^{0}C for 45 min. The suspended material was filtered, the filtrate was evaporated to dryness. The resulted aminopyrrole was dissolved in 3 ml of dry DMF was added to phthalic acid dipentafluorophenyl ester (25 mg, 0.07 mmol), The reaction mixture was stirred for 15 hours at 55^{0}C , DMF was evaporated. The Boc-protected derivative **276** was dissolved in methanol (3 ml) and 3 ml of 4N HCl in dioxane was added. In 30 min the solvent was evaporated and the solid was purified by HPLC as described in example 1, step D. Yield 40% of compound **274**. ES MS: 607.78 ($M+H^+$).

EXAMPLE 139

N,N'-Bis-[5-(2-guanidino-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **277**

A solution of compound **274** (30 mg, 0.05 mmol) and pyrazole-1-carboxamidine hydrochloride (0.1 mmol, 9 mg) in 5 ml of DMF were kept at ambient temperature overnight, evaporated. The residue was purified by HPLC as as described in example 1, step D. Yield 68% of N,N'-Bis-[5-(2-guanidino-ethylcarbamoyl)-1-(3-methyl-butyl)-1H-pyrrol-3-yl]-terephthalamide **277**. ES MS: 691.81 ($M+H^+$).

Formulation Examples

The following are representative pharmaceutical formulations containing a compound of Formula (I).

Example 1

Tablet formulation

The following ingredients are mixed intimately and pressed into single scored tablets.

Ingredient	Quantity per tablet, mg
compound of this invention	400
cornstarch	50
croscarmellose sodium	25
lactose	120
magnesium stearate	5

Example 2

Capsule formulation

The following ingredients are mixed intimately and loaded into a hard-shell gelatin capsule.

Quantity per	Ingredient capsule, mg
compound of this invention	200

lactose, spray-dried	148
magnesium stearate	2

Example 3

Suspension formulation

The following ingredients are mixed to form a suspension for oral administration.

Ingredient	Amount
compound of this invention	1.0 g
fumaric acid	0.5 g
sodium chloride	2.0 g
methyl paraben	0.15 g
propyl paraben	0.05 g
granulated sugar	25.0 g
sorbitol (70% solution)	13.00 g
Veegum K (Vanderbilt Co.)	1.0 g
flavoring	0.035 ml
colorings	0.5 mg
distilled water	q.s. to 100 ml

Example 4

Injectable formulation

The following ingredients are mixed to form an injectable formulation.

Ingredient	Amount
compound of this invention	0.2 mg-20 mg
sodium acetate buffer solution, 0.4 M	2.0 ml
HCl (1N) or NaOH (1N)	q.s. to suitable pH
water (distilled, sterile)	q.s. to 20 ml

Example 5

Suppository formulation

A suppository of total weight 2.5 g is prepared by mixing the compound of the invention with Witepsol® H-15 (triglycerides of saturated vegetable fatty acid; Riches-Nelson, Inc., New York), and has the following composition:

compound of the invention	500 mg
Witepsol® H-15	balance

Biological Examples

Example 1

Minimum Inhibitory Concentration (MIC) Assays:

The assays described below were used to measure the minimum inhibitory concentration (MIC) of a compound necessary to completely inhibit visible growth of the organism tested. These assays are adapted from NCCLS protocols M7-A4 and M27-A (NCCLS vol 17:9 and vol 17:2) as modified by Sandven, S. *Clin. Micro.* (1999) 37:12, p.3856-3859. MIC values for Aspergillus fumigatus were determined using NCCLS protocol M38-P.

Inoculum preparation, incubation and reading results

All compounds were dissolved in 100% DMSO to a stock concentration of 10mM and use fresh or stored at -80 °C. Stock compounds were kept frozen until needed and used freshly with no more then one freeze-thaw cycle. When used for test purposes, compounds were diluted in the appropriate media depending on the organism being tested.

For yeast and aspergillus species, seven 1:2 serial dilutions of compound in appropriate media buffered with MOPS at pH 7.0 were prepared such that the final starting test compound concentrations were 50.0 uM for yeast and 50 uM aspergillus species. For bacteria, dilutions were made in growth media used for the particular bacteria being tested.

Yeast

Five well-separated colonies from a 24hr Sabouraud Dextrose plate incubated at 35C were picked and resuspended into 5.0 ml of normal saline. The $O.D._{530}$ was read and the

culture was adjusted to 0.5 McFarland units with normal saline. A 1:2000 dilution was made with RPMI 1640 media buffered with MOPS at pH 7.0 and 100 μL of this inoculum preparation was added to an equal volume of test compound-containing media. 25 μL of the redox indicator Alamar Blue (Biosource International) was added to each well and the plates were incubated for 48h at 35 C. Wells having yeast growth changed color from blue to pink. Accordingly, the MIC was calculated based on the well with the lowest concentration which did not change color from blue to pink, e.g., growth was inhibited.

Bacteria

Inoculums are made in the same manner as yeast except all dilutions are made in normal saline, with a final dilution of 1:200 and an inoculum of 10 μL. Solid and liquid media, as well as plate incubation times for the various organisms tested, are listed in Table 1 below.

Table 1

Organism	Liquid media	Solid media (agar)	96 well plate incubation time	Definition
VRE-UCD3	BHI	BHIA	No vancomycin –16h 25 μg/mL Vancomycin - 24h	BHI-Brain Heart Infusion
VRE-CSUC4	BHI	BHIA	No vancomycin –16h 25 μg/mL Vancomycin - 24h	BHI-Brain Heart Infusion
VRE-UL17	BHI	BHIA	No vancomycin –16h 25 μg/mL Vancomycin- 24h	BHI-Brain Heart Infusion
VRE-BM4147	BHI	BHIA	No vancomycin –16h 25 μg/mL Vancomycin- 24h	BHI-Brain Heart Infusion
Moraxella catarrhalis	BHI	BHIA	16h	BHI- Brain Heart Infusion

Bacillus cereus	CAMHB	BHIA	16h	BHI- Brain Heart Infusion
Pseudemonas aeruginosa	CAMHB	BHIA	16h	BHI- Brain Heart Infusion
Staphylococcus aureus	CAMHB	BHIA	16h	CAMHB-Cation adjusted Muller Hinton broth
Haemophilus influenzae	HTM	Chocolate Agar	24h	Chocolate Agar-Nutrient agar +5% heat lysed Sheep blood

Streptococcus pneumoniae	CAMHB+ 5% LHB	MHA + 5% SB	24h	LHB-Lysed Horse Blood
Candida albicans	RPMI	SABDEX	48h	SABDEX-Sabouraud Dextrose Agar

Filamentous fungi

Inoculums are made by incubating *Aspergillus fumigatus* for 7 days at 35 C on potato dextrose agar slants. Slants are then covered with 1.0ml of 0.85% saline, one drop of Tween 20 is added and colonies are teased with a sterile transfer loop to create a suspension which is allowed to sit for 5 min so heavier particles can drop out. The upper suspension is separated and adjusted to an optical density of 0.09 to 0.11. The resulting suspension is diluted 1:50 which yields 2X the final inoculum needed. Micro dilution trays are prepared as with yeast and incubated for 48h at 35C. For our purposes the MIC is defined as the lowest compound concentration at which no visible growth is observed after 48h.

Compounds of this invention were tested in assays described above and were found to be active. Examples of compounds that exhibited antibacterial activity (MIC <45.5 μM) are shown in FIG. 5. Examples of compounds that exhibited antifungal activity (MIC <45.5 μM) are shown in FIG. 6.

Topoisomerase Inhibition Assays

Candida albicans topoisomerases I and II (cTop1 and cTop2) were isolated according to Fostel et al. (1992) and Shen et al. (1992). Human topoisomerases I and II (hTop1 and hTop2) were purchased from Topogen (Columbus, OH).

Inhibition of topoisomerase I

Effects of GL compounds on DNA relaxation by topoisomerase I were studied using gel electrophoresis. Negatively supercoiled plasmid DNA (pARG, 8 kb) was used as the substrate. The reaction for *C. albicans* toposiomerase I was performed in 25 mM TrisHCl, pH 7.5, 50 mM NaCl, 2.5 mM MgCl2, 0.5 mM EDTA and 50 ug/mL BSA at 35°C. The reaction was stopped at any given time by adding SDS to a final concentration of 0.5%. Subsequently, proteinase K was added to 250 ug/mL and the mixture was incubated at 60°C for 30 min. The reaction mixture was further extracted with phenol followed by phenol:isoamyl alcohol:chloroform (25:1:24). Samples were loaded on 0.8% agarose gel and subject to electrophoresis using 1X TBE. Different DNA intercalators were used for better gel resolution. Ethidium bromide was sometimes added to both the gel and the running buffer to 0.25 ug/mL. In other cases, chloroquine was added to 0.25 ug/mL to separate the DNA topoisomers.

Inhibition of topoisomerase II

Effects of GL compounds on topoisomerase II were investigated by monitoring decatenation reactions using entangled kinetoplast DNA (Topogen). The decatenation reaction was performed in 10 mM TrisHCl, pH 7.5, 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 0.5 mM ATP. The reaction was stopped at any given time by adding SDS to a final concentration of 1%. Subsequently, proteinase K was added to 250 ug/mL and the mixture was incubated at 60°C for 30 min. The reaction mixture was further extracted with phenol followed by phenol:isoamyl alcohol:chloroform (25:1:24). Samples were loaded on 0.8% agarose gel and subject to electrophoresis using 1X TBE. Ethidium bromide was added to both the gel and the running buffer to 0.25 ug/mL.

DNA Binding Properties of Compounds of this Invention

Fluorescence Studies

When compounds prefer to bind to the minor groove of dsDNA, they induce DNA duplex formation. Hybridization of complementary fluorescently labeled strands brings the two labels, fluorescein and dabcyl, in close proximity, thus quenching the fluorescence of fluorescein. Therefore, this hybridization stabilization assay ("HSA") can be used to measure ligand binding to double-stranded DNA.

The DNA binding properties of several compounds of this invention were investigated by fluorescence spectroscopy. The 11-bp oligo CGA_8G ("FQ11") having fluorescein at the 5′ end on one strand and dabcyl at the 3′ end on the complementary strand was used as the AT-rich ligand binding target. At room temperature, FQ11 remains largely single-stranded in the HEN buffer (10 mM HEPES, pH 7.2, 0.1 mM EDTA and 10 mM NaCl).

Fluorescence was measured at the excitation wavelength of 485 nm and the emission wavelength of 530 nm using a 96-well plate fluoreader (PE CytoFluor® Series 4000). The FQ11 concentration was kept at 5 nM (for duplex concentration) for the binding experiments and varying concentrations of ligands were added. All experiments were performed in duplicate in the HEN buffer at room temperature unless otherwise stated. Standard deviations were calculated based on the duplicate experiments. The fluorescence signal was normalized against the fluorescence in the absence of compounds. Decreasing fluorescence signals with increasing ligand concentrations indicated binding of the ligand to dsDNA. Through this least-square fitting procedure, apparent dissociation constants ($K_{d,app}$) for each compound tested were calculated. The studies demonstrated that compounds of this invention bind to DNA very tightly, with apparent $K_{d,app}$ values below 100 nM for most compounds tested.

Circular Dichroism Studies

Because of the electronic interactions between ligand and DNA, ligand binding can often induce circular dichroism ("CD") signals that are absent when DNA or ligand is alone in solution. DNA binding of compounds of this invention were determined using CD spectroscopy.

All solution conditions were the same as described above. PolydA-polydT was used at 50 μM. CD signal was monitored using a JASCO J-600 CD polarimeter at room temperature. The results showed binding properties that indicated a 2:1 complex. The dramatic CD change in the DNA absorbing region (260 – 300 nm) upon binding of these compounds demonstrated that compounds of this invention induced DNA conformational changes.

DNA Thermal Melting Studies

Interactions between DNA and compounds of this invention were investigated using thermal melting techniques monitored at UV wavelength 260 nm. All investigated compounds showed a stabilization effect on DNA duplex formation.

During melting experiments, 3 uM GCGA3T3CGC (A3T3) oligo duplex was mixed with 6 uM of compound in HEN buffer in a total volume of 200 uL. The UV absorbance was monitored at 260 nm with a Beckman UV spectrophotometer with temperature control. The melting temperature (Tm) where half of the duplex dissociates was determined at relative absorbance of 0.5. The free A3T3 has a Tm of approximately 42°C. With the presence of ligands, the Tm increases. The results indicated compounds of this invention tend to stabilize duplex DNA by binding to the minor groove. Increases in Tm have also been observed for duplex oligo CGATTATTAAGC in the presence of the compound.

The foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.